Does Carbon Distribution and Turnover in (Sub)Alpine Grassland Soils Indicate These Areas May Be Potential Carbon Dioxide Hotspots in the Event of Global Warming?

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1 Abstract

Alpine soils are expected to contain large proportions of labile carbon (C), which may become a further source of atmospheric carbon dioxide (CO_2) in the event of global warming. However, there is currently little data available on C storage and distribution in these soils.

The aim of this project was to determine if C storage and distribution in alpine soils indicate they may be a further source of CO_2 with global warming and investigate which other factors may be important in this event.

Soil core samples were extracted from across grassland elevation gradients of silicate and limestone bedrock in the central Swiss Alps and Spanish Pyrenees, respectively. Additionally, soil samples were extracted from across an elevation gradient in the eastern Swiss Alps, where soil cores had previously been translocated, from a high elevation to a lower elevation, to simulate global warming.

Results showed that labile C proportions in siliceous alpine soils were larger than those of temperate soils, however this was not the case in the limestone bedrock soils. Labile material indicated long C turnover times and soil translocation indicated slow microbial community responses to environmental change. The important influence of factors other than climate, such as bedrock type, may play a more influential role on C storage in alpine soils.

Overall the results of this project indicated it is unlikely that alpine grassland soils will result in strong positive feedbacks to global warming.

2 Zusammenfassung

Alpine Böden können grosse Mengen an labilem organischem Kohlenstoff (C) enthalten, der unter veränderten Klimabedingungen möglicherweise als CO_2 freigesetzt wird. Die Annahme über Menge und Verteilung des labilen C dieser Böden ist derzeit jedoch nur unzureichend belegt.

Das Ziel dieser Arbeit war es, die obige Annahme durch neue Daten aus alpinen Ökosystemen zu verifizieren sowie die zu einer Akkumulation führenden Faktoren zu untersuchen. Dies ist eine Voraussetzung zur Abschätzung des Verhaltens des labilen C unter wärmeren Klimabedingungen.

Zur Untersuchung des labilen C wurden entlang von Höhengradienten in den Alpen (Silikatgestein) und den Pyrenäen (Kalkstein) Bodenproben entnommen. Zusätzlich wurde ein bestehendes Bodentranslokationsexperiment entlang eines Höhengradienten beprobt. Dieses Experiment repräsentiert eine Klimamanipulation.

Der Gehalt an labilem C in silikatischen Böden der Alpen war höher als der in Böden der gemässigten Klimazone. Im Gegensatz dazu gab es keine Zunahme des labilen C entlang des Kalksteingradienten. Labiler C war durch lange Umsetzungszeiten gekennzeichnet. Die Veränderung der mikrobiellen Gemeinschaft im Translokationsexperiment zeigte eine nur langsame Anpassung an die neuen Umweltbedingungen. Es ist davon auszugehen, dass andere Faktoren als das Klima, z.B. Geologie, die Anreicherung von labilem C mit steuern.

Schlussfolgerung: Alpine Böden bilden unter veränderten Klimabedingungen wahrscheinlich keine starke CO_2 Quelle.

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6 Title

Does carbon distribution and turnover in (sub)alpine grassland soils indicate these areas may be potential carbon dioxide hotspots in the event of global warming?

7 Summary

Storage and distribution of carbon (C) in soils may have important implications for future atmospheric carbon dioxide (CO_2) levels as global warming may accelerate CO_2 release from soil organic C (SOC). Alpine soils are expected to contain large amounts of labile (readily degradable) C which may become a further source of atmospheric CO_2 as a result of global warming. These areas could be potential 'hotspots' in the event of global warming, where a hotspot is considered to be an area or region where a change in environmental conditions could lead to potentially large changes in soil organic matter (SOM), and could therefore release further CO_2 into the atmosphere. However, there is little data available on these soils, and understanding of the influence of environmental factors on SOM turnover in these environments is limited. Clarification of which factors play a major role of C storage and decomposition within these alpine environments is important, not only for understanding the processes involved, but also for fine-tuning models to ensure predictions are as accurate as possible when considering climate change effects.

Global warming effects are expected to be more pronounced in these alpine zones in comparison to temperate zones. Previous studies which have focused on the impact of warming in these zones have indicated a corresponding change in the vegetation community. Changes in plant community may have important consequences in soil decomposition processes, and therefore soil C storage, through changes in litter input quantity and quality and consequent alterations in the microbial community. The microbial community is an important aspect of the soil ecosystem and, as they are able to respond rapidly to changes in their environment, may be an important early indicator of how changes in the external environment (i.e. global warming) may alter activity within the soil. Changes in the microbial community may alter decomposition processes and therefore this may result in an alteration in C storage and distribution within the soil. However, while vegetation communities are expected to show an upward migration in mountain zones, the extent and direction of response which may occur in soil microbial communities of alpine grasslands as a result of changing environmental conditions is largely unknown.

The project aim, with respect to the exploration of alpine soils as hotspots to global warming, was to determine labile C proportions in comparison to temperate soils and additionally to determine the quantity, degree of stabilisation and mean residence time (MRT) of SOM in relation to site factors such as temperature, soil pH, vegetation, and SOM structure. To achieve this, sites across an elevation gradient were investigated. Elevation gradients provide an opportunity to examine C content and distribution *in situ* along varying climatic conditions and, therefore, consider long-term effects of climate in a natural environment. Furthermore, to address potential changes in alpine soil microbial communities an elevation gradient was used to investigate (i) differences in soil microbial communities across an elevation gradient of (sub)alpine grassland soils in the Swiss Alps, and (ii) the long-term effect of translocation of soil cores from a higher to a lower elevation site.

To address the probability of alpine soils as global warming hotspots, soil core samples on siliceous bedrock were extracted from five grassland sites along a small alpine elevation gradient from 2285 to 2653 metres above sea level (m asl) in the central

Swiss Alps. After measurement of C distribution and quality in these alpine soils, labile C proportions were compiled with data from previous lower elevation studies to explore labile C trends with elevation. Furthermore, a study of C distribution in Pyrenean limestone grassland soils across a larger elevation gradient of 853-2293 m asl was carried out for comparison of C storage in these soils with previous gradient studies of a similar climate and also to compare to siliceous soil gradient data. Finally, soil samples were extracted from across an elevation gradient of a previous soil core translocation study in the eastern Swiss Alps, where soil cores were translocated from an elevation of 2525 m asl to a lower elevation of 1895 m asl to simulate climate change, to investigate changes in soil microbial communities.

From previous studies, the hypothesis for the limestone and silicate grassland gradients of this study was that SOC contents would not indicate any trend with elevation, while labile C proportions would be higher in (sub)alpine soils than in temperate grassland and would indicate an increasing trend with elevation. The hypothesis with respect to the translocated cores was that after longer than a decade, soil microbial community in translocated cores would differ from that at the original site but resemble the community at the new site. Furthermore, it was hypothesised that soil microbial communities would indicate trends with elevation.

Across the siliceous soil alpine grassland small elevation gradient of this study, soil fractions obtained by size and density fractionation revealed a high proportion of labile C in SOM, mostly in the uppermost soil layers. Combined with data from the previous temperate grasslands, these silicate grassland soils did indicate a significant increase in labile C proportion with elevation, however no trend was indicated with SOC content. In contrast, the limestone grassland soils indicated that SOC contents, from 20-cm deep soil, increased significantly with elevation while labile C proportions indicated no trend with elevation. Therefore the indication with C quality and quantity is that high labile SOC proportions are not a general attribute of high elevation mountain soils *per se* but may depend on factors such as geology.

Root biomass, across this limestone grassland elevation gradient, showed a general increase with elevation and root age, estimated by means of radiocarbon dating, significantly increased with elevation. Across the small siliceous soil alpine gradient, MRTs of fine earth, measured by means of radiocarbon dating and turnover modelling, were long compared to those of temperate soils and indicated a general increase between fractions of growing stability. Depending on elevation and pH, plant community data suggested considerable variation in the quantity and quality of litter input, and these patterns could be reflected in the dynamics of soil C. Indeed, chemical composition of soil fractions confirmed a direct relationship of SOM composition to MRT.

Results from soil phospholipid fatty acid (PLFA) analysis confirm significant differences in microbial communities between sites. The soil core translocation from a higher to lower elevation indicated a shift in total microbial biomass (TMB) and proportional distribution of structural groups towards the lower elevation community. Patterns related to translocation were also observed as shifts in the fractional biomass of ectomycorrhizal and arbuscular fungi, and in relative contents of several structural groups. However, even after more than a decade of translocation in their new site, the shift in TMB was only significant in the lower 10 cm and not in the upper 10 cm. Overall, soil microbial community activity and diversity indicate a moderate shift towards new site conditions after 11 years and therefore this data from translocated cores suggest slow responses of microbial communities to environmental changes in alpine soils.

Slow microbial activity is also supported by the long turnover times indicated in the siliceous soils of the small alpine elevation gradient and increasing root turnover with increasing elevation across the temperate to alpine limestone grassland gradient. While

temperature is likely to be a major cause for the slow turnover rate observed, other factors such as litter quality and soil pH, as well as the combination of all factors, play an important role. Ignoring this interplay of controlling factors may impair the performance of models to project SOM responses to environmental change.

8 Introduction

8.1 Background

Concerns with global warming and effects of C emissions are topics which have been greatly debated in the media and scientific communities in recent years. Recent reports on climate change predict that global surface temperature will increase in the range of 1.1-6.4 °C by the end of this century (IPCC, 2007). These changes are expected to be more pronounced in alpine (high elevation areas, above the tree line) environments (Figure 1), with temperature increases of 4-5 °C projected in the Alps by 2050 (SAEFL, 2005), and indeed rising temperatures have already been reported in the European Alps (Beniston *et al*., 2006). It is been suggested that soils in these regions may contain large amounts of labile C due to unfavourable conditions for decomposition (Baritz *et al*., 2004). Alpine soils may therefore be hotspots for SOM loss under changing climate conditions, where a hotspot is defined as a region where potential SOM losses or gains are large (Baritz *et al*., 2004) and this could lead to large increases of CO_2 into the atmosphere. However, there is little data available on C distribution in alpine soils and while studies have indicated that increased warming in the European Alps could result in increased CO_2 release into the atmosphere (Schindlbacher *et al.*, 2009; Hagedorn *et al.*, 2010), how grassland soils, specifically, may respond to global warming is still uncertain.

Figure 1. Global warming effects are predicted to be more pronounced in alpine areas. This picture, taken in 2007, illustrates an alpine grassland slope exposed to sunlight at the Furka pass site in the Swiss Alps.

Elevation gradients provide an opportunity to measure ecosystem properties in different climates in a steady state environment. Each elevation can be used to represent a change in air temperature and long-term effects of global warming can be considered. A previous study of soil fractions across an elevation gradient, carried out by Trumbore *et al*. (2006), indicated an increase in low density (labile) C turnover of 20 cm deep soil with increasing elevation. This increase in C turnover with decreasing MAT has also been reported in heavier soil fractions of forest soils (Hakkenberg *et al*., 2008). However, data on C turnover of alpine grassland soils is lacking and this would be valuable to consider the implications of global warming on these soil ecosystems.

Previous mountain ecosystem studies have focused on the plant community as an early indicator of how soil ecosystems may alter in response to global warming and corresponding shifts in the vegetation community due to warming have been reported (Harte *et al*., 2006; Saleska *et al*., 2002). However, how the soil microbial communities in such cold environments may respond, not only to any direct effect on the soil environment induced by global warming, but also to corresponding changes in the plant community has been less documented. Microbes play an important role in soil ecosystems, influencing processes such as soil formation (Rillig and Mummey, 2006), litter decomposition (Hattenschwiler *et al*., 2005) and C cycling (Hogberg *et al*., 2001).

Therefore, modification in the soil microbial community may be an important early indicator of a change to the soil ecosystem processes which could subsequently alter soil C distribution in response to global warming.

8.2 Storage worldwide

Globally, soils store more than twice as much C as the atmosphere and thrice that of vegetation (Batjes and Sombroek, 1997; Schlesinger and Andrews 2000), with the atmosphere-soil annual C-exchange estimated at around 50-60 Pg (Schlesinger and Andrews, 2000). Atmosphere-soil C interactions may be strongly influenced by global warming (Friedlingstein *et al*., 2006; Jones *et al*., 2005) through effects on both CO_2 assimilation by vegetation (primary production) and CO_2 release by ecosystem respiration. Yet, it remains uncertain whether, in response to raising temperatures, the net feedback effect of SOM will be positive or negative (Reth *et al*., 2009).

Global SOC to a depth of 1 m has been estimated at 1500 Pg (Jobaggy and Jackson 2000); however, as the interaction of factors determining this content is complicated, it is unknown how this C stock will respond to changing environmental conditions caused by global warming. While studies have indicated that climate plays an influential role in current SOC content and distribution (e.g. Post *et al*., 1982; Ganuza and Almendros, 2003; Alvarez and Lavado, 1998; Garcia-Pausus *et al*., 2007), the importance of other factors, for example land-use (Osher *et al*., 2003; Zhang and Zhang, 2009), parent material (Spain, 1990; Leifeld *et al*., 2005), vegetation properties such as biomass (Tian *et al*., 2008) and plant species (Vinton and Burke, 1997; Finzi *et al*., 1998), and physical soil properties such as texture (Arrouays *et al*., 1995; Percival *et al*., 2000) and acidity (Kemmit *et al*., 2006; Foreid *et al*., 2007), have also been shown. This complex interaction of abiotic and biotic factors complicates predictions of how C stocks may change in future years. However, identifying which areas may contain readily degradable C sources (i.e. possible C hotspots) and therefore a potential source of further CO_2 release is an important step in considering the impact that climate change may induce in C stocks worldwide.

8.3 C storage and distribution in alpine soils

Soils in colder environments, such as arctic and alpine tundra that cover large areas in the northern hemisphere, may be of particular concern with respect to global warming as these regions are expected to be more strongly effected than temperate regions (Meehl *et al*., 2007; Rebetez and Reinhard, 2008). Alpine soils cover roughly 4 x 10^6 km^2 worldwide (Körner, 2003), but despite this large extent there is currently insufficient information available with respect to factors influencing SOM transformation in these soils. In temperate soils, C in more transformed mineral associated fractions comprises most of the total soil C (Zimmermann *et al*., 2007), whereas the limited data available from alpine tundra soils (Leifeld *et al*., 2009; Neff *et al*.,

Figure 2. Alpine grassland slope and mountain background, taken at time of sampling (October 2007), at Furka pass site in the Swiss Alps.

2002; Wang *et al*., 2008) suggest large SOC contents and a comparatively high abundance of less decomposed, labile C material in alpine soils.
Studies have indicated that climate change is more pronounced in mountain areas (Figure 2), compared to temperate zones (Meehl *et al*., 2007; Rebetez and Reinhard 2008). While climate change is expected to result in an alteration in the snow melt period, extension of the vegetation period (Bavay *et al*., 2009; Beniston *et al*., 2003) and an upward migration of vegetation zones (Walther *et al*., 2005) it is unknown how SOC contents will respond.

Exploring total and labile C distribution along elevation gradients provides an opportunity to consider the effect of variation in climate on this characteristic, as mean annual temperature (MAT) varies naturally with elevation. While gradient studies cannot replace but only complement ecosystem manipulation experiments or modelling for predicting future situations, they provide a unique opportunity to study long-term effects of major environmental variables on the state of soils (Figure 3). Recent gradient studies have focused on C content and distribution (Garcia-Pausas *et al*., 2007; Yang *et al*., 2008) while exploration of patterns in SOM of varying stability has also been included in some studies (Zimmermann *et al*. 2007; Leifeld *et al*., 2009 Wang *et al*., 2005; Wang *et al*., 2008; Djukic *et al*., 2010b). However, information on both C content and distribution in addition to SOM states of stability are generally lacking in alpine areas and are essential to determine which processes are important in predicting the effect of global warming in alpine SOM.

Figure 3. Subalpine grassland slope and landscape view from sampling site at 1817 m asl, taken in June 2008, at Berguedà in the Spanish Pyrenees.

8.4 SOM stability processes

SOM, a heterogeneous mix of plant, animal and microbial residues existing in various states of microbial decomposition has been shown to contain fractions of varying stability and different turnover times ranging from a few years to centuries (Wang *et al*., 2005; Baisden *et al*., 2002). Studies have shown that stabilisation and turnover of SOM is influenced by properties of the physical soil environment, such as O_2 availability (Bunnell *et al*., 1977), soil texture and mineralogy (Balesdent *et al*., 1988; Feller and Beare, 1997), soil pH and soil aggregation (Tisdall and Oades, 1982; Cambardella and Elliott, 1994). Furthermore, the influence of the vegetation community through litter input quality (Melillo *et al*., 1982; Stump and Binkley, 1992) in addition to soil microbial activity (McGill, 1996), have also been identified as important factors in SOM turnover.

The mechanisms of SOM stability in temperate soils have been identified and generally accepted by the scientific community. These have been described as (i) selective preservation, (ii) spatial inaccessibility and (iii) mineral surface interactions (Sollins *et al*., 1996). The process of selective preservation results in recalcitrant molecule accumulation through molecular characteristics of SOM which are resistant to microbial degradation. Spatial inaccessibility is the process whereby soil microbes are unable to

physically access SOM within the soil matrix, this can occur, for example, through SOM occlusion in soil aggregates. Mineral surface interactions can prevent microbial decomposition processes through sorption or bonding of SOM to mineral surfaces. While the contribution of these interacting SOM stability processes on SOM decomposition has been discussed in temperate soil (von Lützow *et al*., 2006) it is likely that they also play an important role in determining SOM stabilisation of alpine soils and thus modulate the influence of the harsh climate. However, the role of such mechanisms in alpine soils has scarcely been explored thus far and both the proportional distribution of fractions within SOM and their degree of stabilisation are important in consideration of which mechanisms are important in these areas.

8.5 POM fractions and stability

Fractions of different stability can be separated by size and density fractionation (Balesdent *et al*., 1998; Buyanovsky *et al*., 1994) into particulate organic matter (POM) fractions which have been shown to be particularly sensitive to changing environmental conditions (Cambarella and Elliott, 1992). POM fractions are related to the mechanisms of selective preservation and physical accessibility as described above. A free POM (fPOM) fraction is made up of partially decomposed litter and is thus closely related to the amount and quality of incoming plant litter. The amount of litter input depends on vegetation productivity (Meentemeyer *et al*., 1982; Körner, 2003), whereas litter quality is largely determined by plant species and tissue type (Kögel-Knabner, 2002), particularly in ecosystems with slow transformation rates (Hobbie, 2000). More transformed material, known as occluded POM (oPOM), is encapsulated in soil aggregates and is therefore expected to be less readily accessible to soil microbes. The remaining heavy fraction is the mineral associated matter (mOM) portion of SOM. The mOM fraction is the most transformed, and therefore the least accessible to further microbial decomposition due to its physically bound state; studies have indicated that mOM is older than POM fractions (Sollins *et al*., 1996; Leifeld *et al*., 2009).

When conditions are conducive for microbial decomposition, as this process proceeds, organic compounds derived from plants are increasingly replaced by those derived from microbes (Berg and Meentemeyer, 2002). Initial breakdown of plant polysaccharides decreases O-alkyl-C and increases alkyl-C (Kölbl and Kögel-Knabner, 2004). Thus, the alkyl-C/O-alkyl-C ratio, which increases from light/coarse to fine/heavy soil fractions, can be used as an indicator of the degree of decomposition of SOM fractions (Golchin *et al*., 1994a; Helfrich *et al*., 2006). Currently, it is unknown whether alpine POM has a similar or an even lower degree of transformation compared to temperate fractions, and how the chemical composition of SOM in alpine environments relates to its turnover rate. These characteristics may also provide a valuable indication of which factors play a key role in SOM decomposition in alpine ecosystems.

8.6 Factors previously shown to influence alpine SOM content and turnover

SOM quantity and quality is mainly determined by two processes: (i) primary productivity and the consequent net input and (ii) decomposition rate. The partitioning of C between the different fractions, which predetermines the sensitivity of SOM to environmental changes, is highly spatially variable as it varies depending on edaphic conditions, land use history and current management (John *et al*., 2005; Grandy *et al*., 2009).

MRT of labile C at high elevations exceeds that of temperate soils (c. 90-170 years vs. 10 years, Leifeld *et al*., 2009). This increase in MRT with elevation, and the associated accumulation of labile C in mountain soils, has mainly been attributed to decreasing

temperature (Trumbore *et al*., 1996; Wang *et al*., 2005). However, it has been suggested that other factors such as strong soil acidity may also play a crucial role (Leifeld *et al*., 2008). Furthermore, plant diversity in alpine ecosystems have previously been shown to enhance soil aggregate stability (Pohl *et al*., 2009), as discussed above, this has implications for SOM stability processes through spatial accessibility. Moreover, litter quality from alpine plant species may differ greatly from that at lower sites and this may also affect the composition of SOM. While it is unclear which of these fractions are most influential in alpine soils, previous studies have explored this question through a number of studies to investigate or simulate climate change.

8.7 Climate change impact on terrestrial ecosystems - indications from previous studies

8.7.1 Soil warming

Studies have indicated that rising atmospheric CO_2 levels will result in higher global mean temperatures and consequently, to soil warming and an upward migration of vegetation zones in mountain regions (Dullinger *et al*., 2003; IPCC, 2007). The combined influence of these climate and vegetation changes, along with the subsequent variation in litter input, may induce a large change in soil microbial community composition which, in turn, affects nutrient cycling.

Soil warming experiments can reveal information on the response of microbial communities with respect to direct soil warming. A short-term study based on PLFA analysis indicated that a rise in soil temperature increased the pool size of substrate C available for microbial respiration through a compositional and functional shift in microbial community (Zogg *et al.,* 1997). Longer-term studies have indicated that soil warming reduces microbial biomass and alters microbial community composition (Frey *et al.,* 2008) with microbial respiration eventually acclimatising over time after an initial increase (Luo *et al.,* 2001; Bradford *et al.*, 2008). Not-sustained warming effect on respiration were observed by Elliason *et al*. (2005) and Vicca *et al.* (2009) and interpreted as indicative for exhaustion of available substrate. A long-term study of soil communities in a subarctic heath indicated that a time period of over ten years was necessary to detect significant responses to warming, shading and fertiliser addition (Rinnan *et al*., 2007). Since the conditions in alpine soils vary considerably between seasons (Lipson and Schmidt, 2004), mainly due to the effect of variable periods of snow cover and snow melt, microbial communities in these cold soils could be expected to show high resilience to a change in environment. This characteristic, combined with lower annual temperatures and consequently lower activity in alpine soils, may result in a slow response in microbial community dynamics compared to temperate and Mediterranean soils. However, the complex interplay of abiotic and biotic variables in alpine regions, in addition to the variation in these factors between alpine regions worldwide complicates the predictions of the widespread impact of global climate change in these areas worldwide. Furthermore, as global warming involves not only changes in air and soil temperatures, but also in precipitation, soil moisture and vegetation, the complex interactions of these various factors should be considered when assessing potential influences of future climate change.

8.7.2 Elevation gradients

Elevation gradients have previously been utilised as indicators of global warming as climate varies naturally with elevation and it is therefore possible to consider how the interplay of all factors involved in global warming impacts the properties of the environment being studied. Garcia-Pausas *et al*. (2007) carried out a study across a Pyrenean chain grassland elevation gradient of 1845-2900 m asl. They reported large

SOC contents of 20-30 kg m^{-2} in some of their sites under strongly different lithology, however, there was no trend in C stocks with elevation in this gradient. In contrast, Yang *et al*. (2008) showed an increase in C stock with elevation for Tibetan grasslands. Additionally, labile C such as POM has also been shown to increase with elevation. This trend has been reported in temperate to alpine grasslands in siliceous Swiss alpine soils (Zimmermann *et al*., 2007; Leifeld *et al*., 2009) and across an elevation gradient of 1700-3900 m asl, with varying parent material, in Gongga mountain soils of Tibet (Wang *et al*., 2005). An increase in POM with elevation may be related to increasing root densities (Leifeld *et al*., 2009). For grasslands, it has not been evaluated hitherto whether an increasing proportion of soil labile C with elevation is restricted to more acidic soils or is also typical for soils on limestone. This seems particularly relevant as soil acidity acts as a co-variable of temperature along elevation gradients in the siliceous Alps and has been discussed as an important driver for SOC turnover times in mountain grasslands (Leifeld *et al*., 2008).

A primary study of grassland soils in the Swiss Alps revealed increasing labile C content with elevation from 880 to 2200 m asl (Leifeld *et al*., 2009). The largest proportion of labile C was indicated at the highest elevation site, above the timberline, where it comprised 86 % of total SOC in the top 0-5 cm, but only 24-61 % in soils at lower elevations. As this study only involved a single alpine site, further sampling of siliceous grassland alpine sites is necessary to determine if this is a general characteristic of these soils or if this was an exceptional site. If large proportions of labile C are a general characteristic of alpine soils then this indicates a readily degradable source of C that could further increase atmospheric CO_2 levels.

8.7.3 Soil translocation

Translocation experiments on gradients provide an opportunity to simulate environmental change *in situ* and, consequently, to examine long-term effects of combinations of abiotic and biotic factors within a natural environment. The response of soil microbial community composition to changes in vegetation was investigated by translocation of soil cores between an open grassland and an adjacent environment under an oak canopy (Waldrop and Firestone, 2006). Only 2 years after transplantation of the soil cores into their new environment, microbial community composition indicated a shift from oak to grassland soil, but not vice versa. The authors speculated that the response of the microbial community depended on the degree of variation of the environment experienced at the original site, suggesting that microbial communities in systems experiencing a greater variation within that environment throughout the year are more resilient than those of more stable environments. Waldrop and Firestone (2006) observed that changes in microbial community, as a result of a shift in plant community induced by core translocation, occurred rapidly and within the first few years in soil of a Mediterranean climate.

Data from a translocation study across an elevation gradient in the Swiss Alps indicated reductions in the above and below-ground phytomass after 3 years for cores moved from cold to warmer sites, but microbial communities were not measured at that time (Egli *et al*., 2004). Soil cores remaining from this translocation study (Figure 4) provide an opportunity to measure the long-term changes in the soil microbial communities of alpine soils which may occur from increased air temperature and the corresponding changes in vegetation community. While site, vegetation and soil characteristics are already available from previous data published from the soil core translocation study (Egli *et al*., 2004, Hitz *et al*., 2001), remaining translocated cores, along with samples from other sites along the gradient can be characterised by PLFA analysis to provide information on the soil microbial communities.

While soil microbial communities have been shown to respond rapidly (within hours) to changing climate conditions (Linn and Doran, 1984), studies carried out *in situ* have

indicated slow responses of soil microbial communities, for example Balser and Firestone (2005) reported no significant change in the microbial biomass of soil cores 2 years after they had been translocated from a higher elevation of 1200 m asl to a lower elevation of 400 m asl. In contrast, Djukic *et al*. (2010a) reported a rapid response of microbial biomass over a 2 year period in soil cores which were translocated from an alpine grassland site of 1900 m asl to lower elevation sites at 1300 and 900 m asl. Thus, studies have shown that changes in soil microbial communities are complex and therefore, how soil microbial communities may respond to future environmental changes is still largely unknown and requires further investigation (Balser and Wixon, 2009; Frey *et al*., 2008). Examination of soil microbial community trends with elevation and any modifications induced through translocation, to simulate climate change, would provide valuable information in considering the impact an altered environment may have on soil microbial communities in alpine zones.

Figure 4. Translocation site of soil cores at Vereina valley from *Egli *et al*. study carried out in 1997 and 2000. Photo indicates location of remaining translocated cores at Vereina site and was taken in July 2008.

Egli et al. 2004. J. Plant Nutr. Soil Sci. 167: 457-470.

9 Aims and objectives

9.1 Determine C storage and distribution in alpine grassland soils

The study started with the premise that the previously observed accumulation of labile C at high elevations needs to be confirmed by data from different sites. In addition, data for C distribution among SOM fractions, their turnover time and sensitivity to site conditions should help to improve predictions of the response of SOM in alpine soils to environmental changes. Using a collection of soil samples from across a small elevation gradient of an alpine grassland, the 1st aim of this study was to determine:

(1) Are labile C proportions of siliceous alpine grassland sites larger than those reported in temperate grasslands, i.e. are alpine grasslands potential C hotspots?

(2) Does labile C proportion indicate a trend with elevation?

(3) What does C distribution and stability indicate with respect to the productivity of these alpine sites?

9.2 Does C storage increase with elevation in limestone grassland soils?

Compilation of siliceous alpine grassland soils with data of siliceous grassland soils previously measured along a lower elevation gradient enables the exploration of any trends with elevation in this soil type. However, SOC storage and distribution along a similar elevation of limestone bedrock was also measured to determine if any trends with elevation are a general observation of grassland soils or whether soil type/parent material may be an important contributing factor. The 2nd aim of this study was to measure total and labile SOC contents across a Pyrenean limestone grassland elevation gradient to determine if:

(1) SOC contents increase with elevation across this limestone grassland gradient

(2) Labile C proportions increase with elevation from temperate to alpine limestone grasslands

9.3 Explore which factors are involved in C storage and distribution of alpine soils

When considering alpine soils as potential hotspots, varying sampling sites along elevation gradients enables consideration of the effect of climate change in these soil systems. With respect to C storage, how the distribution, degree of stabilisation and turnover of SOC relates to elevation was investigated. Additionally, whether these soil C characteristics indicate any relationship with other soil ecosystem variables such as plant species distribution, litter quality and physical and chemical soil properties was explored.

9.4 What is the long term response of alpine grassland microbial communities to change in environmental conditions?

The final aim of this study was to investigate differences in soil microbial communities between (sub)alpine grassland sites with different environmental conditions, and the long-term effect of translocation. In this translocation study, the aims were to:

(1) Investigate the long-term effect of translocation of soil cores from a higher to a lower elevation site

(2) Evaluate soil microbial communities trends with elevation across a gradient of (sub)alpine grassland soils in the Swiss Alps

9.5 Hypotheses

Labile C Proportions

As previous studies in temperate siliceous grassland soils indicated increasing labile C proportion with elevation (Leifeld *et al*., 2009; Zimmermann *et al*., 2007), the hypothesis was as follows:

(1) Compared to lower elevation soils, alpine siliceous grassland soils would indicate large proportions of labile C, which increased with elevation

(2) Labile C proportion would increase with elevation from temperate to alpine sites across a limestone grassland elevation and comprise more than 30% of total SOC at elevations above 2000 m asl

Total C contents

From data published in previous studies of limestone grasslands (Garcia-Pausas *et al*., 2007) and siliceous grasslands (Leifeld *et al*., 2005), the hypothesis was that SOC contents would not indicate any trend with elevation across the elevation gradients in either soil type.

Soil Microbial communities

As a previous study indicated an alteration in below-ground phytomass after 3 years translocation (Egli *et al*., 2004), the hypothesis was that after longer than a decade of translocation:

(1) Soil microbial communities in translocated cores would be different from that at the original site but would not yet have reached the structure of the new site

Furthermore with respect to examination of microbial communities across an elevation gradient:

(2) Soil microbial communities would indicate trends with elevation

10 Site details

10.1 Small elevation siliceous soil grassland alpine gradient: Furka pass

As land management has been shown to affect the content of SOC and its distribution (Chan, 2001; Yamashita *et al*., 2006), sites with homogenous bedrock (silicate) and management (sheep grazing) were selected to minimise variations due to management and geology. Samples were collected in October 2007 from sites on a westerly facing slope (Figure 5) in an alpine pasture area with low-intensity grazing sheep near the Furka pass in the central Swiss Alps (Figure 6; Ellipsoidal WGS84, Lat 46°56´ N, Long 8°4´ E).

To create an elevation gradient, five sampling sites varying with elevation were selected: 2285, 2379, 2481, 2564 and 2653 m asl. The aspect of all sites was the same and the average slope inclination was 35°. ^{137}Caesium measurements (data not shown) and visual inspection of the soil profiles indicated no or negligible soil erosion.

Figure 5. View overlooking the lowest sampling sites, 2379 and 2285 m asl, of the alpine siliceous grassland elevation gradient at Furka pass in the Swiss

Figure 6. Map locations of the siliceous grassland elevation gradient sites, Furka pass and Vereina valley, in the Swiss Alps. Image constructed in Google Earth.

10.2 Limestone grassland soils elevation gradient: Berguedà

Grassland sites (853-2293 m asl) were selected from the Pyrenees area of Berguedà, Catalonia, Spain (Figure 7; Ellipsoidal WGS84, lat 42°16´ N, long 1°41´ E; lat 42°15´ N, long 1°41´ E; lat 42°13´ N, long 1°46´ E; lat 42°13´ N, long 1°50´ E) after consultation with the owners/ farmers to obtain grazing history of the locations before sampling. Soil core samples were collected along an elevation transect with limestone bedrock in June 2008 from four sites of the following elevations: 853, 1279, 1817 and 2293 m asl (Figure 8). Historical and current management of all sites were identified as low-intensity sheep grazing pastures, although there may also be recent low-intensity horse grazing at site 1279 m.

Figure 7. Location of the limestone grassland elevation gradient sites in the Berguedà area of Catalonia, Spain. Image constructed in Google Earth.

Figure 8: Grassland sampling sites, at 853 m asl (left) and 1279 m asl (right), of Berguedà limestone elevation gradient, taken at time of sampling in June 2008.

10.3 Soil core translocation elevation gradient: Vereina valley

Sites from the Vereina valley location (Figure 6; Ellipsoidal WGS84, lat 46°50´ N, long 9°58´ E; lat 46°49´ N, long 9°59´ E; lat 46°46´ N, long 9°59´ E) were used as the second Swiss grassland, silicate bedrock gradient in this study. Sampling sites included the sites of the previous soil translocation study carried out in 1997 on grassland soils of the eastern Swiss Alps (Egli *et al*., 2004). The whole region is extensively grazed during a few weeks in summer, mainly by cattle. The natural timberline is at around 2100 m asl but sites below that were deforested centuries ago. Egli *et al*. (2004) extracted soil cores in 1997 (diameter 7 cm, maximum lengths 50 cm including living vegetation) from a high elevation (colder) alpine tundra site (Jöri: 2525 m asl, *Curvuletum* community) and relocated these to a lower elevation (warmer) subalpine grassland site (Vereina Valley: 1895 m asl, *Nardetum* community with interspersed *Carex fusca*) to simulate climate change (Figure 9). Cores were wrapped in a soft, fine-meshed plastic net and placed in exactly fitting bore-holes at the new site. Within-site translocation at the Jöri site indicated no change in above- or below-ground plant biomass due to extraction itself during the first three experimental years (Egli *et al*., 2004), however, there were no remaining within-site translocated cores available for retrieval during the sampling in 2008. In contrast, above-ground biomass and roots significantly declined within the first three years of translocation at their new site, indicating a vegetation shift to the new environment. The elevation change in Egli *et al*. (2004) corresponds to a difference in air MAT of 3.3 °C but peak topsoil temperature in summer may differ by up to 15 °C (Egli *et al*., 2004). Sites also show a strong elevation gradient in season length, litter input, and root turnover (Hitz *et al*., 2001). A summary of basic site properties of all three gradient locations used in this study are shown in Table 1.

Figure 9. View looking up towards the highest elevation site (Jöri) at the Vereina valley grassland elevation gradient in the Swiss Alps.

11 Methods

11.1 Sampling location climate details

Weather data was taken from the Swiss hydrological atlas and extrapolated by means of a climate model to determine the mean annual air temperature (MAT) and mean annual precipitation (MAP) for the Furka pass sampling location (Schwarb *et al*., 2001; Z'graggen, 2002). Additionally, soil temperature loggers (Onset, Hoboware, USA) were inserted at 5 and 10 cm depths at the highest and lowest sites to determine variation in soil temperature across the gradient. Loggers were removed after 13 months (2008/2009) and the mean soil temperature over one year was calculated.

At Berguedà MAT and MAP values were determined for each site as per Ninyerola *et al*. (2005) while at the Vereina valley site MAT and MAP values were taken from Gabathuler (1999) and EDI (1992), respectively.

11.2 Sampling and bulk soil separation

11.2.1 Furka pass

Six 30 cm soil cores (core diameter: 7.7 cm; Figure 10) were extracted at each site along a 20 m horizontal transect. Each soil core was cut into sections representing the following depths: 0-5, 5-10, 10-20 and 20-30 cm. Sections were oven dried at 40 °C before analysis.

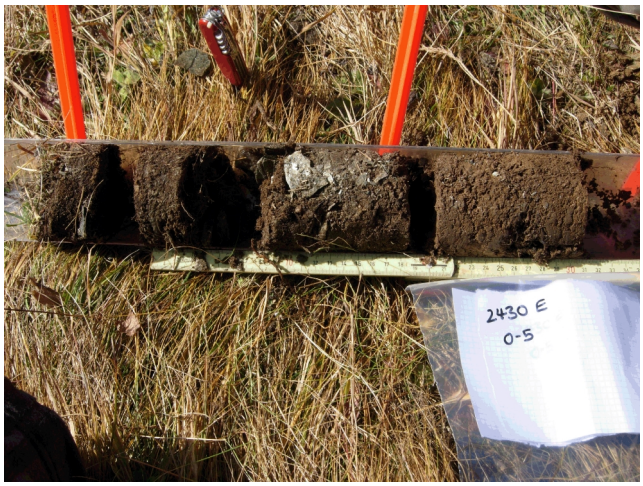


Figure 10. Siliceous grassland soil core replicate from site 2481m asl at Furka pass gradient. Photo taken at time of sampling in October 2007, after separation of the core into: 0-5, 5-10, 10-20 and 20-30 cm sections.

Above ground plant material (phytomass) was cut from the topsoil sections of each core. Plant litter present in the upper core sections was removed by hand along with larger stones and roots. The remaining soil sample was sieved to obtain smaller stone content (>2000 µm), which was added to the larger stones to obtain total stone content, and the fine earth section (<2000 µm). As fine earth still contained some fine roots (and litter material in the upper sections) this section was sieved further to obtain very fine earth material of < 63 µm (which did not contain any root material) and the root/litter containing larger fine earth material of 63-2000 µm. This additional fine root/litter material contained in the 63-2000 µm fine earth section was separated by flotation in water to obtain a total root/litter fraction. The root/litter free 63-2000 µm fine earth section was then added to the < 63 µm to achieve a total fine earth section (0-2000 µm). Larger hand picked root material was added to flotation obtained root/litter material to obtain total root/litter material. After separation into phytomass, root/litter, fine earth and stones, each fraction was weighed.

11.2.2 Berguedà

Eight 20 cm deep soil core replicates (core width x breadth: 5 cm x 5 cm; Figure 11) were extracted at each site as four dual replicates (paired cores with a distance of c. 1 m to each other). The horizontal distance among these sampling pairs was 10 to 100 m. Each soil core was cut into 2 sections representing depths of 0-10 and 10-20 cm. Sections were oven dried at 40 °C before analysis.

Figure 11. Limestone grassland soil core replicate at the time of sampling from site 853 m asl at Berguedà gradient, prior to separation into different depths.

Larger stones and root material were removed from dried soil samples by hand before the remaining sample was sieved to obtain total stone content (>2000 µm) and the fine earth section (<2000 µm). To separate the root material from the fine earth section it was sieved further to obtain very fine earth material of < 63 µm (which did not contain any root material) and the larger fine earth material of 63-2000 µm which still contained some fine root material. This additional fine root material contained in the 63-2000 µm fine earth section was separated by flotation in water, washed, and oven dried as previously. The root free 63-2000 µm fine earth section was oven dried separately and added to the < 63 µm section to achieve a total fine earth section (0-2000 µm). Larger hand picked root material was added to the dried flotation obtained root material to result in a total dry root matter fraction. Stone, dry root matter and fine earth fractions were all weighed separately to calculate fraction densities.

11.2.3 Vereina valley

The three remaining translocated soil cores at the Vereina Valley site (1895 m asl) were extracted in September 2008, i.e. after 11 years. Sample replicates were also collected from the soil immediately surrounding the translocated cores for comparison. In addition soil replicates were collected from the site that the cores were originally extracted from (Jöri site, 2525 m asl; Figure 12) and from a lower elevation site (Stutzegg: 1665 m asl, Nardetum community) that had also been included in the earlier study to expand the range of

Figure 12. Soil core replicate at the time of sampling from Jöri site (2525 m asl) at Vereina valley gradient, prior to separation into different depths.

environmental conditions (Figure 13). Soil samples were separated into 0-10 cm and 10-20 cm depths for analysis. After collection, samples were kept cool in ice boxes at all times during transportation to prevent changes in the soil microbial community then stored overnight in a freezer at -20 °C. All samples were freeze dried over night before separation of root and stone material (>2 mm) by dry sieving; remaining root material was removed from fine soil (<2 mm) by hand before soil samples were ball-milled. Samples for PLFA analysis were kept frozen at -20 °C until further analysis.

Figure 13. Grassland sampling sites at Stutzegg: 1665 m asl (left) and Jöri: 2525 m asl (right) at the Vereina Valley elevation gradient in the Swiss Alps. Photos taken at time of sampling in September 2008.

11.3 Fine earth analysis and calculations

11.3.1 Physical soil properties

11.3.1.1 Bulk densities (Furka pass and Berguedà)

Sample stone volumes were calculated by applying a stone density of 2.65 g cm^{-3} with the measured stone weights. Fine earth bulk densities (g cm^{-3}) were calculated by dividing fine soil weight by total core volume minus stone volume for all sites and depths.

11.3.1.2 Texture (Furka pass and Berguedà)

Soil texture of composite samples from all replicates at each site and depth were determined by the pipette method after removal of SOM with H_2O_2 (Gee and Bauder, 1986). N.B. Not all 0-5 cm depth Furka pass samples contained enough material for analysis because of their high SOM content.

11.3.2 Chemical soil properties

11.3.2.1 Soil pH (Furka pass, Berguedà and Vereina)

To determine pH values of the fine earth, samples were mixed with 0.01 M $CaCl_2$ solution (2.5:1 dilution). Soil pH of all replicates at each site and depth were measured then site pH values were calculated from the mean average of replicates at each depth.

11.3.2.2 Nutrient content (Furka pass and Berguedà)

Extractable soil nutrient concentrations (potassium (K), calcium (Ca), magnesium (Mg) and phosphorous (K)) of fine earth were determined according to the Swiss reference

methods from dual composite replicates for all sites and depths after treatment with 1:10 NH_4-acetate solution (FAL, 1998).

11.3.2.3 C and N concentrations (Furka pass, Berguedà and Vereina valley)

Furka pass and Vereina fine earth sample replicates from all sites and depths were milled with a ball-mill and analysed for C and nitrogen (N) concentrations after combustion using an elemental analyser (Hekatech Euro EA 3000, Wegberg, Germany). As Berguedà fine earth samples contained carbonate, milled samples were treated by acid fumigation with hydrochloric acid, to remove inorganic C, before C and N concentrations were measured, as previously.

11.3.2.4 SOC contents (Furka pass and Berguedà)

C and N concentrations were used to calculate C/N ratios for fine earth from all sites and depths. C concentrations were used with fine earth bulk densities to determine SOC contents (kg m^{-2}). Site values were calculated as the mean of all replicates.

11.4 Root properties and calculations (Furka pass and Berguedà)

Root weight was used together with the core surface area to determine dry root content in t ha^{-1} for both gradients. Furka pass root samples from all sites, depths and replicates were milled with a ball-mill and analysed for C and N concentrations after combustion using an elemental analyser, as with fine earth samples, then used to calculate root C/N ratios. N.B. The upper layers of the Furka pass site also contained some litter material which could not be separated from root material and therefore the Furk pass samples are referred to as root/litter material. Berguedà root samples were compiled from all site replicates to obtain composite samples for each site and depth which were then measured for C and N concentration then C/N ratios were calculated, as with Furka pass root samples.

11.5 Soil fractionation, POM separation and analysis (Furka pass and Berguedà)

Furka pass and Berguedà fine earth samples from all sites and depths were separated by density fractionation into free POM (fPOM) and occluded POM (oPOM), these fractions represent the labile portion of SOM.

Fine earth sample replicates from all sites and depths were centrifuged with 1.8 g cm^{-3} sodium polytungstate (SPT) solution until all heavy (pellet) material was separated from light (floating) material. All floating material (≤ 1.8 g cm^{-3}) was collected as fPOM in a 20 µm sieve, washed thoroughly and oven dried overnight at 60 °C. The remaining pellet was re-suspended in SPT and treated with ultra sonification (22 J ml^{-1}) to destroy aggregates before repeated centrifugation and collection of oPOM (≤ 1.8 g cm^{-3}). This method was used to correspond with the fractionation method used by Zimmerman *et al*. (2007) and Leifeld *et al*. (2009) to allow direct comparison of the POM fraction values. All POM fractions were checked with an electrical conductivity meter after washing to ensure that any remaining salt solution was low and certainly below a level which could interfere with further analysis of the POM material.

Both fPOM and oPOM material were ball-milled and each fraction was measured for C and N concentrations with an elemental analyser; C/N ratios for each POM fraction and total (fPOM C plus oPOM C) particulate organic C (POC) content were calculated as with fine earth. Mineral associated C (MOC) content and C/N ratios for mOM, which in this case is the remaining heavy (>1.8 g cm^{-3}) mineral fraction, were calculated by

difference. The soil proportion of labile C and mineral associated C were calculated (in %) from POC and MOC content relative to total SOC content, respectively.

11.6 Calculation of C turnover times (Furka pass and Berguedà)

11.6.1 Bomb model

Nuclear weapon tests in the 1960's released additional radioactive C (^{14}C) into the atmosphere, which, when taken up by vegetation, is incorporated into SOM through decomposition and mineralisation of plant derived litter. The increase in isotopic signature of SOM provides an opportunity to measure the C residence time (Harkness *et al*., 1986). So called 'bomb models' are used to relate the proportion of ^{14}C in SOM to the level of ^{14}C in the atmosphere during the last several decades, assuming a steady state system (i.e. the same amount of C enters the soil on an annual basis). The latter assumption is often questioned but is seems reasonable for alpine ecosystems as they occur naturally without a history of land use change. Combined with chemical analysis of SOM, ^{14}C dating can indicate whether or not the chemical structure controls turnover of plant residues.

11.6.2 Radiocarbon measurement (Furka pass and Berguedà)

POM fractions, fine earth and washed root/litter material from the Furka pass site were selected for ^{14}C measurement by accelerator mass spectrometry (AMS) at the radiocarbon laboratory at the ETH Zurich University. Fractions were selected, depending on quantity of sample material available, to cover variability of C allocation and composition in the soil. Three soil fractions (fPOM, oPOM, mOM by difference) from 5-10 cm were used from the two lowest and highest sites, and site 2564 m asl was chosen for a detailed profile over four depths as this site had a particularly large amount of labile sample material (root/litter/POM).

Site variability of MRT of fPOM was investigated with six horizontal sample replicates (5-10 cm) from two sites (2564 m and 2285 m). Replicates with similar SOC contents were selected, and 3 dual replicate combinations were pooled for each site. To obtain an overview of bulk soil turnover rates across the entire gradient, samples of fine soil using all six replicates from each soil depth were pooled.

Washed upper root material from each elevation of the Berguedà gradient was combined and measured for ^{14}C as with Furka pass samples. N.B. As Berguedà fine earth samples were found to contain some very old black C material, fine earth samples could not be used to obtain reliable soil C MRT estimates

11.6.3 Time-lag application to bomb model calculations (Furka pass)

MRT was estimated by means of ^{14}C dating. AMS measurement of ^{14}C yielded percentage data of modern C (pMC) which were then inserted into the bomb model to obtain estimates of MRT for each SOM fraction (all pMC values are given in Appendix 1). A detailed description of the model can be found in Harkness *et al*. (1986), and an application to soil fractions in Leifeld and Fuhrer (2009).

MRTs were calculated for root/litter fraction composite samples from all four depth sections within the 0-30 cm core, taken from site 2564 m. Root/litter MRTs ranged from 12.5-15.5 years and did not indicate any trend with soil depth. A mean value of 14.5 years was derived from these values and used in the bomb model to recalculate soil MRT to account for the period of time that C remains in plants before it enters the first fraction in the soil decomposition process. In this case, it is referred to as the time-lag period and the adjusted model is referred to as the bomb model with time-lag. All pMC

values were inserted into the bomb model with time-lag to recalculate MRT of each SOM fraction. Corresponding fine earth MRTs for these fractions were calculated by using fraction specific MRTs in conjunction with SOC contents to provide a weighted average according to Leifeld and Fuhrer (2009). These fine earth MRTs, later referred to as 'fraction calculated fine earth MRT', should provide a more accurate estimate of the fine earth MRTs as the contribution from each soil fraction to the total soil turnover is integrated into the calculation.

11.6.4 Fraction contribution to MRT calculations (Furka pass)

For many samples, only fine earth radiocarbon data were available. MRT calculated for fine earth samples may be biased as the calculation treats SOC erroneously as a homogenous pool (Trumbore *et al*., 1997). In order to improve the MRT estimates, a regression approach was applied using fraction calculated fine earth MRT. First, composite fine earth MRT estimates were determined from the pooled samples from all elevations and depths by inserting pMC values into the bomb model with time-lag, as previously with soil fractions. Measured composite fine earth MRTs were then plotted against their corresponding fraction calculated fine earth MRTs to determine the relationship between MRT estimated by these two different methods. This equation was then applied to the measured composite fine earth MRTs to adjust for the relative contribution of each fraction of varying stability and to obtain a more reliable MRT estimate of the fine earth for all elevations and depths. The following curved relationship was identified:

y = -1272+658*LOG10(x); r = 0.96 (1)

Where y = fraction calculated fine earth MRT and x = measured composite fine earth MRT.

Calibration encompassed composite fine earth MRTs of between > 70 and 2000 years and therefore was not applied to shorter MRTs. For measured composite fine earth MRT values of 70 years or shorter, the original composite fine earth MRT was kept. This was the case for 0-5 cm depth samples at all elevations and the 5-10 cm samples for site 248l m. Therefore, for these fine earth samples of shorter MRT, the time-lag period is still accounted for however a recalculation according to Eq. (1) is not appropriate.

11.6.5 C input rates (Furka pass and Berguedà)

Annual C input into the soil at each depth for each site were calculated from SOC contents divided by MRTs. Total site productivity was indicated by the total annual C input into the 30 cm cores.

C input by roots (t C ha^{-1} a^{-1}) was calculated as the ratio of standing root C over root MRT.

11.7 Chemical composition analysis

Milled fine earth, POM fraction and root/litter samples were selected for ^{13}C NMR spectroscopy analysis to correspond with those already measured for ^{14}C content for comparison, individual sample analysis was not replicated due to cost restrictions. The ^{13}C chemical shifts in ^{13}C CPMAS NMR spectra (Bruker DSX 200 NMR spectrometer, Bruker, Karlsruhe, Germany; resonance frequency 50.32 MHz, contact time 1.0 ms, pulse delay 150 ms, magic angle spinning speed 6.8 kHz) were measured relative to tetramethylsilane (0 ppm) (^{13}C NMR spectra of soil fractions are shown in the Appendix 2).

Chemical groups from CPMAS ^{13}C NMR spectra were categorized by division of the spectra into 4 regions: Alkyl-C (-10 – 45 ppm), O-Alkyl-C (45 – 110 ppm), Aryl-C (110 – 160 ppm) and Carboxyl-C (160 – 220 ppm) (Knicker and Lüdemann, 1996). For studying the relationship between SOM composition and turnover time, chemical group concentrations were compared with MRTs. The most significant relationship determined from this comparison was indicated from a narrowed O-alkyl region of 60 – 90 ppm, which excludes protonated C of lignins (resonance around 110 ppm) as well as methoxyl-C (resonance around 56 ppm). Integration peaks in each region were used to calculate the relative distribution (%) of each chemical group within the sample measured. Alkyl-C/O-Alkyl-C ratios were then calculated for each soil fraction as an indicator of microbial transformation (Baldock *et al*., 2007).

11.8 Vegetation (Furka pass)

11.8.1 Above-ground phytomass

Above ground phytomass was calculated (in g m^2) from the plant weights obtained from the soil cores. Median values were used as sampling area (0.00465 m^2) was relatively small and a single plant stem could significantly bias the mean of the six replicates per site.

11.8.2 Species identification and functional group classification

At each site, plant species were identified and percentage area (25 cm x 25 cm) distribution was estimated by a modified Braun-Blanquet method at the location of each sampled core. At each site, total number of species was counted, and each species was categorized into one of the following functional groups: lichens, sedges, grasses, forbs, legumes and dwarf shrubs. Relative abundance (%) of each functional group was calculated.

11.8.3 Ellenberg indicators

This information was applied to the Ellenberg's indicator system (Ellenberg, 1988) to identify plant community characteristics. Ellenberg values indicate the ecological niche for environmental factors and can characterize ecological conditions based on plant species prevalence (Hawkes *et al*., 1997; Ersten *et al*., 1998). Ellenberg indicator values are expressed relative to the environmental factor on a 9 point scale where low values correspond to a low value for that particular factor. For example, when considering temperature, lower values indicate a plant community preference for colder temperatures while higher values correspond to warmer temperature preference, while with respect to soil acidity, lower values indicate preference for soils of low acidity while higher values correspond to a vegetation community preference for soils of less acidity/higher alkalinity. At each of the sites on the Furka pass elevation gradient Ellenberg indicator values were calculated for light, temperature, soil moisture, pH and soil nutrient status.

11.9 Microbial measurement by PLFA analysis (Vereina valley)

11.9.1 PLFA analysis as measurement of soil microbial biomass

It has been estimated that a large majority (80-99%, Amann *et al*., 1995) of soil microorganisms cannot be cultured by conventional methods. Phospholipid fatty acid (PLFA) analysis provides an opportunity for a more complete overview of the viable microbial community *in situ*. PLFAs are a structural component of cell walls and

decompose rapidly after cell death and therefore, extraction, identification and quantification of fatty acids from soil samples provide a profile of the living soil microbial community (White *et al*., 1979). Modifications of PLFA profiles indicate a change in the soil microbial community (Lovell *et al*., 1995; Frostegård *et al*., 2006) while individual PLFAs can act as indicators as they are specific to certain microbial groups (Guckert *et al*., 1985; Degrood *et al*., 2005; Wilke *et al*., 2004). In addition, the ratio of specific PLFAs in relation to others has been shown to indicate changes in microbial community structure due to external factors such as nutrient limitation, osmotic stress etc (Knivett and Cullen, 1965; Guckert *et al*., 1986).

11.9.2 Lipid extraction

Soil PLFA contents were extracted from fine earth of each replicate from all sites and depths according to a modified Bligh and Dyer (1959) and White *et al*. (1979) method then measured by gas chromatography combustion mass spectrometry (GC-C-MS) on a JandW DB-5 capillary column (50 m x 0.10 mm x 0.33 µm), as described in Paterson *et al*. (2007). PLFA were quantified relative to the C19:0 internal standard and expressed in PLFA weight in µg per g soil.

11.9.3 Nomenclature, ratios and chemical groups

PLFA nomenclature follows Frostegård *et al*. (1993a). Total PLFA concentration has previously been used to indicate total microbial biomass (TMB) of the soil (Frostegård *et al*., 1993a; White *et al*., 1996; Wu *et al*., 2010). Cyclopropane fatty acids (cyFA) cy17:0 and cy19:0 have been identified as indicators for Gram(+) anaerobes (Vestal and White, 1989; Ratledge and Wilkinson, 1988). Fatty acids with a 10Me designation represent the actinomycetes group (Frostegård *et al*., 1993b; Evgrafova *et al*., 2008; Kelly *et al*., 2003). The PLFA 18:2ω6,9 has been used as an indicator for ectomycorrhizal (EM) fungi (Olsson, 1999; Djukic *et al*., 2010a) while 16:1ω5*c* and 18:1ω7 indicate arbuscular mycorrhizal (AM) fungi (Haack *et al*., 1994; Olsson, 1995). Gram(+) bacteria can be indicated by the terminally branched saturated PLFAs (Ponder *et al*., 2009). The PLFA relative abundance of cyFA (cy17:0 + cy19:0)/ monoenoic precursors (16:1ω7*c* + 18:1ω7*c*) is an indication of nutrient stress (Guckert *et al*., 1986; Kief *et al*., 1994).

Individual PLFAs were divided into chemical groups according to structure and then the contribution of each group to the total biomass was calculated as a percentage. PLFAs were divided into one of the following five groups: terminally branched saturated (tbS), mid-chain-branched saturated (mbS), straight-chain saturated (scS), mono-unsaturated (mUS) and polyunsaturated (pUS).

11.10 Statistics

11.10.1 Furka pass

Soil and root characteristics were calculated as the mean of the six replicates with standard errors (SE) at each site, with the exception of phytomass for which median values were used. Effects of factors site and soil depth on amount and distribution of SOM were tested by one-way ANOVA and, if significant at the 5% error probability, a post-hoc Tukey's test was applied. Data were log-transformed where they did not pass tests of homogeneity of variances or normality of distribution. Correlation between selected variables is expressed as Pearsons' correlation coefficient and an indication of the error probability. These analyses, regression analysis, and the curved relationship between composite bulk soil MRTs and fraction calculated bulk soil MRTs in equation (1) were determined using Statistica 9.0.

11.10.2 Berguedà

Soil and root characteristics were calculated as the mean of the eight replicates with SE at each site. In exception to this are root C/N ratios, where a composite sample from all site replicates was measured in duplicate and soil nutrients and texture, where the mean of 4 dual replicate measurements were used to calculate the mean and SE. Regression analysis and one-way ANOVAs between site variables were determined using Statistica 9.0. Correlation between selected variables is expressed as Pearsons' correlation coefficient and an indication of the error probability. Dependent t-test was used to compare bulk densities between 0-10 and 10-20 cm depths. All replicates were used for correlation analysis between variables whereas correlation between soil variables and elevation were determined using mean values (i.e., n = 4).

11.10.3 Vereina Valley

Soil core measurements were calculated as the mean of the three replicates with the SE at each site. One-way ANOVA analysis and Tukey's HSD multiple comparison tests were applied to soil TMB, chemical group proportion and biomarker proportion to test for significant differences between sites and soil depths using Statistica 9.0. Fatty acid profiles using concentrations of single fatty acids (% of total PLFA) were subjected to principal component analysis.

12 Results

12.1 Sampling location climate details

At the Furka pass location, calculated average MAT and MAP were 0 °C and 1890 mm respectively (Table 1), with monthly mean temperature ranging from -7.3 °C in February to 6 °C in August. Mean annual soil temperature at 5 and 10 cm depths varied little between the lowest and highest sites and was 2.8/2.6 °C at 2285 m and 2.7/2.9 °C at 2653 m respectively. The soil type was identified as dystric cambisol (spodic) developed on mica schist (WRB, 2006).

Table 1. Elevation, climate, soil type and land management details for each sampling gradient location.

Location	Elevation gradient (m asl)	MAT (°C)	MAP (mm)	Soil type (WRB)	Land management
Furka pass (Swiss Alps)	2653-2285	-1 – 1.1	1890	dystric cambisol	sheep grazing
Berguedà (Pyrenees)	2293-853	3.9 – 10.6	816-917	rendzic leptosol	sheep grazing
Vereina valley (Swiss Alps)	2525-1665	-2.2 – 2.3	1600-2000	haplic podzol	cattle grazing

At the Berguedà location, MAT ranged from 3.9 – 10.6 °C across all sites and decreased with elevation while MAP indicated no trend with elevation. The soil type was identified as clayey rendzic Leptosol developed on limestone (WRB 2006).
At the Vereina valley location, MAT ranged from -2.2 – 2.3 °C (Table 1: Gabahuler, 1999), also decreasing with elevation. Soils at all sites are haplic podzols (Hitz *et al*., 2001).

12.2 Fine earth properties

12.2.1 Physical soil properties (Furka pass and Berguedà)

Across the Swiss alpine grassland small elevation gradient, bulk densities decreased significantly with soil depth at all sites ($p < 0.05$) but did not indicate any trend with elevation (Table 2). Soil clay content varied from 1-24 % across all sites and depths, decreased with depth at all sites and averaged at 10 % in the 30 cm cores across all sites. Across the Pyrenean limestone grassland gradient, bulk densities increased with depth at all sites although this decrease was only significant at site 1279 m asl ($p < 0.05$). While 0-10 cm bulk densities decreased highly significantly with elevation ($r = 1.00$; $p < 0.01$) the 10-20 cm bulk densities trend with elevation was not significant ($r = 0.86$; $p > 0.05$; however, the site bulk densities (0-20 cm) still indicated a significant decrease with elevation ($r = 0.95$; $p < 0.05$) across the gradient. Clay comprised the highest proportion of the fine earth but did not indicate any trend with depth; across all sites the average clay proportion was 44 %, followed by 32 % and 24 % in silt and sand, respectively.

Table 2. Furka pass and Berguedà: Physical soil properties of fine earth at each sampling site and soil depth.

Site location	Site elevation (m asl)	Soil depth (cm)	Bulk density (g cm^{-3})	SE	Clay (%)	Silt (%)	Sand (%)
Furka pass (Swiss Alps)	2285	0-5	0.33	0.08	14	27	59
		5-10	0.74	0.05	10	31	59
		10-20	0.73	0.04	9	33	59
		20-30	0.9	0.03	9	29	63
		0-30	*0.72*	*0.02*	*10*	*30*	*60*
	2379	0-5	0.23	0.03	-	-	-
		5-10	0.45	0.06	17	33	50
		10-20	0.56	0.04	10	34	57
		20-30	0.72	0.02	6	32	62
		0-30	*0.54*	*0.02*	-	-	-
	2481	0-5	0.36	0.06	-	-	-
		5-10	0.64	0.05	6	29	65
		10-20	0.74	0.07	1	33	66
		20-30	0.85	0.03	6	31	63
		0-30	*0.70*	*0.04*	-	-	-
	2564	0-5	0.25	0.02	-	-	-
		5-10	0.51	0.03	16	33	51
		10-20	0.62	0.01	8	33	59
		20-30	0.65	0.04	5	30	65
		0-30	*0.55*	*0.01*	-	-	-
	2653	0-5	0.51	0.07	24	24	52
		5-10	0.67	0.02	11	27	62
		10-20	0.76	0.03	6	27	66
		20-30	0.90	0.03	7	26	67
		0-30	*0.75*	*0.03*	*10*	*26*	*63*
Berguedà (Spanish Pyrenees)	853	0-10	1.13	*0.06*	45	*31*	*24*
		10-20	1.34	*0.05*	41	*35*	*24*
		0-20	*1.24*	*0.03*	*43*	*33*	*24*
	1279	0-10	1.01	*0.03*	37	*32*	*31*
		10-20	1.49	*0.09*	38	*39*	*23*
		0-20	*1.23*	*0.05*	*37*	*35*	*27*
	1817	0-10	0.81	*0.03*	52	*29*	*20*
		10-20	0.99	*0.05*	54	*32*	*14*
		0-20	*0.90*	*0.03*	*53*	*30*	*17*
	2293	0-10	0.68	*0.02*	44	*28*	*28*
		10-20	0.87	*0.02*	43	*31*	*26*
		0-20	*0.77*	*0.02*	*44*	*29*	*27*

(SE) indicate 1 SE of the mean from 6 replicates at Furka pass, 8 at Berguedà and 3 at Vereina valley

12.2.2 Chemical soil properties (All sites)

The Furka pass alpine grassland soils were found to be strongly to moderately acidic with pH values ranging from 3.9 to 5.5, which did not indicate any consistent trend with soil depth or trend with elevation, with the least acidic pH at the middle elevation (Table 3). Soil at the Vereina valley (sub)alpine and alpine soils were also found to be strongly acidic, also indicating no trend with depth or elevation. The Pyrenean limestone grassland soil pH's were comparatively more neutral, ranging from 6.0 – 7.4 across all sites and depths, but not vary significantly with depth ($p > 0.05$). Site pH (0-20 cm) generally decreased with elevation, although this trend with elevation was not significant ($r = -0.89$; $p > 0.05$) and soil acidity was still only modest at the highest elevation site.

Across the small Swiss (Furka pass) siliceous alpine gradient, fine earth OC concentrations ranged from 108-279 (g kg^{-1}) in the 0-5 cm sections and decreased sharply with soil depth at all sites (Table 3). OC concentrations did not indicate any trend with elevation while concentrations at sites 2379 and 2564 m asl were significantly higher ($p < 0.05$) than the other 3 sites. Fine earth N concentrations ranged from 5.8-15.5 (g kg^{-1}) in the 0-5 cm sections and mirrored the trends of the OC concentrations. Site C/N ratios were highest at the top elevation site and lowest at the lowest elevation site but did not increase significantly with elevation ($p > 0.05$) due to lower C/N ratios at the middle site.

Across the Spanish limestone grassland soils, site fine earth OC concentrations varied from 26.7–77.9 g kg^{-1} across the elevation gradient, increased significantly with elevation ($r = 0.97$; $p < 0.05$) and individual OC concentrations decreased significantly with depth in the upper three elevation sites ($p < 0.05$). Individual fine earth N concentrations also shared this pattern of significant decrease with depth across the specified sites and site N concentrations also indicated a significant increase with elevation ($r = 0.95$; $p < 0.05$). Individual fine earth C/N ratios generally decreased with depth but not significantly ($p > 0.05$) at any site. While site C/N ratios generally increased with elevation, this increase was not significant ($r = 0.92$; $p > 0.05$).
Across the Vereina Valley elevation gradient the Swiss grassland soil pH's varied between 3.5 and 4.2, with lowest values at the Vereina site. While soil pH did not vary significantly between sites ($p > 0.05$), values in the translocated cores were closer to their site of origin than their new environment in both soil depths.

OC concentrations in the fine earth decreased with depth at the Jö ri and Stutzegg sites and in the Jö ri-Vereina cores, however not at the Vereina site, where the concentrations were significantly ($p < 0.01$) higher at both depths than at the other sites. C/N ratios were lowest at the lowest elevation site and increased with soil depth at all sites; ratios were highest at the 10-20 cm depth of the Vereina site. Both OC concentrations and C/N ratios in the Elevation cores were still similar to their site of origin in the 0-10 cm sections but different from the site of translocation, although the OC concentrations were slightly higher in the lower soil layer.

At Furka pass, nutrient concentrations (K, Ca, Mg and P) were highest in the 0-5 cm layer where most roots occurred, and decreased steeply with soil depth (Table 4). The highest elevation site contained the lowest concentration of soil nutrients. Site fine earth nutrient concentrations did not indicate any trend with elevation but were generally, with the exception of K, higher at the two lower elevation sites.

In general, the Berguedà limestone gradient soil nutrient concentrations were lower than those of the siliceous grassland sites, except for magnesium concentrations. Across all sites P and K concentrations varied significantly with depth ($p > 0.05$) while Ca and Mg did not ($p > 0.05$).

Table 3. Furka pass, Berguedà and Vereina valley: Chemical soil properties of fine earth at each sampling site and soil depth.

Site location	Site elevation (m asl)	Soil depth (cm)	Fine earth pH_{CaCl2}	OC (g kg^{-1})	SE	N (g kg^{-1})	SE	C/N ratio	SE
Furka pass (Swiss Alps)	2285	0-5	4.3	118	11.0	7.6	0.6	15.5	0.5
		5-10	4.0	39.4	4.1	2.9	0.3	13.8	0.2
		10-20	4.2	17.3	1.4	1.4	0.1	12.0	0.3
		20-30	4.4	11.4	1.9	1.0	0.2	11.3	0.3
		0-30	*4.3*	*25.0*	*3.6*	*1.9*	*0.1*	*13.2*	*0.1*
	2379	0-5	4.6	279	47.3	15.5	0.8	18.1	0.6
		5-10	4.1	114	22.0	7.5	0.9	15.2	0.4
		10-20	4.2	43.6	7.1	3.0	0.2	14.4	0.3
		20-30	4.4	25.4	4.2	1.6	0.1	15.8	0.3
		0-30	*4.3*	*58.0*	*15.3*	*3.7*	*0.3*	*15.9*	*0.3*
	2481	0-5	5.2	129	28.6	8.6	0.4	15.0	0.2
		5-10	4.6	37.9	7.7	2.7	0.4	13.9	0.3
		10-20	4.5	19.3	3.7	1.5	0.2	13.2	0.3
		20-30	4.4	12.2	2.5	1.1	0.1	11.5	0.4
		0-30	*4.6*	*26.0*	*8.1*	*1.9*	*0.2*	*13.5*	*0.1*
	2564	0-5	4.0	242	13.8	13.3	0.5	18.0	0.5
		5-10	3.7	98.2	8.2	6.8	0.4	14.5	0.5
		10-20	4.0	42.7	1.9	2.8	0.2	15.2	0.5
		20-30	4.3	33.4	3.0	1.8	0.2	18.5	0.5
		0-30	*4.1*	*57.0*	*5.3*	*3.5*	*0.1*	*16.3*	*0.3*
	2653	0-5	4.0	108	22.0	5.8	0.8	18.6	0.8
		5-10	4.2	42.9	9.6	2.6	0.4	16.5	0.4
		10-20	3.9	20.6	4.0	1.4	0.2	15.0	0.3
		20-30	4.4	12.9	2.4	0.8	0.1	15.4	0.7
		0-30	*4.2*	*29.0*	*7.4*	*1.8*	*0.2*	*16.6*	*0.4*
Berguedà (Spanish Pyrenees)	853	0-10	7.4	32.3	2.2	3.2	0.2	10.1	0.3
		10-20	7.4	22.3	2.0	2.3	0.2	9.7	0.4
		0-20	*7.4*	*26.7*	*1.0*	*2.7*	*0.1*	*9.9*	*0.3*
	1279	0-10	7.3	51.9	2.5	4.4	0.2	11.8	1.2
		10-20	7.3	13.0	0.6	1.2	0.1	10.8	0.7
		0-20	*7.3*	*28.3*	*1.5*	*2.6*	*0.1*	*10.9*	*0.5*
	1817	0-10	7.1	67.3	5.5	5.9	0.6	11.4	0.4
		10-20	7.1	50.8	4.8	4.1	0.4	12.4	0.5
		0-20	*7.1*	*58.3*	*5.0*	*4.9*	*0.5*	*11.9*	*0.4*
	2293	0-10	6.0	100	3.1	8.2	0.3	12.2	0.1
		10-20	6.3	60.4	3.1	5.3	0.4	11.4	0.3
		0-20	*6.1*	*77.9*	*3.1*	*6.6*	*0.3*	*11.8*	*0.2*
Stutzegg (Swiss Alps)	1665	0-10	3.6	82.0	8.3	7.1	0.7	11.7	0.7
		10-20	3.7	52.0	5.6	3.5	0.2	14.8	1.3
Vereina	1895	0-10	3.5	413	9.8	22.3	0.5	18.5	0.1
		10-20	3.5	450	50.8	15.7	2.8	29.0	1.1
Jöri-Vereina cores	1895	0-10	3.7	132	29.0	8.1	2.0	16.4	0.3
		10-20	4.0	67.0	2.0	3.5	0.3	19.3	0.5
Jöri	2525	0-10	3.7	131	24.7	7.8	1.4	16.7	0.2
		10-20	4.2	43.0	3.2	2.2	0.2	19.3	0.2

(SE) indicate 1 SE of the mean from 6, 8 and 3 replicates at Furka pass, Berguedà and Vereina valley, respectively

Table 4. Furka pass and Berguedà: Nutrient concentration of fine earth at each sampling site and soil depth.

Site location	Site elevation (m asl)	Soil Depth (cm)	Ca (g kg^{-1})	SE	P (mg kg^{-1})	SE	K (mg kg^{-1})	SE	Mg (mg kg^{-1})	SE
Furka pass (Swiss Alps)	2285	0-5	11.6	-	76	-	558	-	222	-
		5-10	2.5	-	24	-	133	-	63	-
		10-20	0.8	-	5	-	42	-	12	-
		20-30	0.5	-	3	-	24	-	5	-
		0-30	2.9	-	*25*	-	*152*	-	*62*	-
	2379	0-5	38.9	-	111	-	946	-	726	-
		5-10	9.3	-	39	-	258	-	228	-
		10-20	1.7	-	6	-	72	-	46	-
		20-30	0.5	-	2	-	21	-	8	-
		0-30	5.5	-	*24*	-	*173*	-	*135*	-
	2481	0-5	33.5	-	59	-	596	-	424	-
		5-10	8.5	-	13	-	117	-	135	-
		10-20	4.3	-	6	-	67	-	68	-
		20-30	2.0	-	3	-	40	-	28	-
		0-30	10.3	-	*17*	-	*160*	-	*163*	-
	2564	0-5	12.7	-	133	-	747	-	350	-
		5-10	2.3	-	44	-	169	-	90	-
		10-20	0.6	-	7	-	40	-	17	-
		20-30	0.3	-	4	-	23	-	5	-
		0-30	1.3	-	*29*	-	*127*	-	*61*	-
	2653	0-5	6.1	-	45	-	261	-	150	-
		5-10	1.6	-	16	-	82	-	53	-
		10-20	0.6	-	4	-	26	-	12	-
		20-30	0.4	-	2	-	11	-	5	-
		0-30	0.9	-	*17*	-	*86*	-	*50*	-
Berguedà (Spanish Pyrenees)	853	0-10	*62*	15	*12*	*2.7*	*237*	13	*315*	*41*
		10-20	*63*	16	*5.3*	*1.2*	*176*	17	*282*	*51*
		0-20	*63*	*15*	*8.1*	*1.7*	*203*	*13*	*296*	*46*
	1279	0-10	*84*	2.1	*9.8*	*1.1*	*185*	8.3	*383*	*7.0*
		10-20	*94*	0.5	*1.9*	*0.1*	*103*	6.2	*365*	*11*
		0-20	*91*	*1.0*	*5.2*	*0.5*	*138*	*7.4*	*378*	*10*
	1817	0-10	*7.4*	0.6	*7.3*	*0.7*	*271*	13	*152*	*15*
		10-20	*6.3*	0.5	*2.5*	*0.3*	*194*	17	*110*	*17*
		0-20	*6.8*	*0.5*	*4.7*	*0.5*	*228*	*13*	*130*	*16*
	2293	0-10	*4.0*	0.3	*9.9*	*0.8*	*384*	90	*184*	*7.3*
		10-20	*3.6*	0.6	*2.6*	*0.3*	*193*	43	*93.9*	*5.7*
		0-20	*3.8*	*0.4*	*5.8*	*0.5*	*277*	*63*	*134*	*5.9*

(SE) indicate 1 SE of the mean from 4 dual composite replicates at Berguedà and were not possible for Furka pass samples as individual composite samples were measured

12.3 C storage and distribution (Furka pass and Berguedà)

At Furka pass, total SOC content (0-30 cm) varied from 5.5 to 10.2 kg m^{-2} across sites and were higher at 2379 and 2564 m than at the other sites and decreased with soil depth (Table 5). Labile C % also decreased with soil depth, in contrast to mineral associated C % which increased. Highest labile C proportions in the range of 71.2-85.5 % were found in the uppermost layers (0-5 cm) and low values between 6.3-19.1 % in the lower depths (20-30 cm). Cumulated over the upper 30 cm, the middle site had the highest relative abundance of labile C while sites 2379 and 2564 had both the highest C concentrations and largest stocks.

Table 5. Furka pass: SOC content, labile and mineral associated C proportions at each sampling site and soil depth. Different lower case letters indicate significant differences for individual soil depth across sites and different capital letters indicate significant differences between sites for values 0-30 cm (Tukeys', $p < 0.05$).

Site location	Site elevation (m asl)	Soil depth (cm)	SOC (kg m^{-2})	SE	Labile C proportion (%)	SE	Mineral associated C proportion (%)	SE
Furka pass (Swiss Alps)	2285	0-5	1.8 a	0.3	71.2 a	3.5	28.8 a	3.5
		5-10	1.4 a	0.1	45.8 ab	4.6	54.2 ab	4.6
		10-20	1.2 a	0.1	20.7 a	2.9	79.3 a	2.9
		20-30	1.0 a	0.2	10.4 a	2.6	89.6 a	2.6
		0-30	*5.5 A*	0.2	*25.6 AB*	1.8	*74.4 AB*	1.8
	2379	0-5	3.2 c	0.2	85.4 a	4.6	14.6 a	4.6
		5-10	2.4 b	0.2	66.6 b	9.0	33.4 b	9.0
		10-20	2.4 b	0.2	20.7 a	2.8	79.3 a	2.8
		20-30	1.8 b	0.1	11.9 ab	2.9	88.1 ab	2.9
		0-30	*9.8 B*	0.4	*25.1 AB*	2.4	*74.9 AB*	2.4
	2481	0-5	2.1 ab	0.2	83.8 a	2.6	16.2 a	2.6
		5-10	1.1 a	0.1	49.4 ab	7.0	50.6 ab	7.0
		10-20	1.4 a	0.1	23.4 a	2.6	76.6 a	2.6
		20-30	1.0 a	0.1	14.3 ab	1.2	85.7 ab	1.2
		0-30	*5.6 A*	0.4	*29.6 B*	1.6	*70.4 B*	1.6
	2564	0-5	2.9 bc	0.2	85.5 a	2.7	14.5 a	2.7
		5-10	2.5 b	0.1	26.1 a	3.7	73.9 ac	3.7
		10-20	2.6 b	0.1	16.0 a	1.4	84.0 a	1.4
		20-30	2.1 b	0.1	19.1 b	1.7	80.9 b	1.7
		0-30	*10.2 B*	0.2	*20.5 A*	1.4	*79.5 A*	1.4
	2653	0-5	2.5abc	0.2	77.3 a	9.0	22.7 a	9.0
		5-10	1.4 a	0.3	26.9 a	3.8	73.1 a	3.8
		10-20	1.5 a	0.2	14.6 a	2.0	85.4 a	2.0
		20-30	1.2 a	0.1	6.3 a	0.3	93.7 a	0.3
		0-30	*6.7 A*	0.6	*19.7 A*	1.6	*79.3 A*	1.6

(SE) indicate 1 SE of the mean from 6 replicates

Across the temperate to alpine Pyrenean limestone gradient, site SOC contents varied from 6.5–11.9 kg m^{-2} (Figure 14) and increased significantly with elevation across the

gradient (r = 0.98; p < 0.05). As with OC and N concentrations, individual SOC contents were higher in 0-10 than in the 10-20 cm layer and decreased significantly with depth at the upper three elevation sites (p < 0.05) but not the lowest site (p > 0.05).

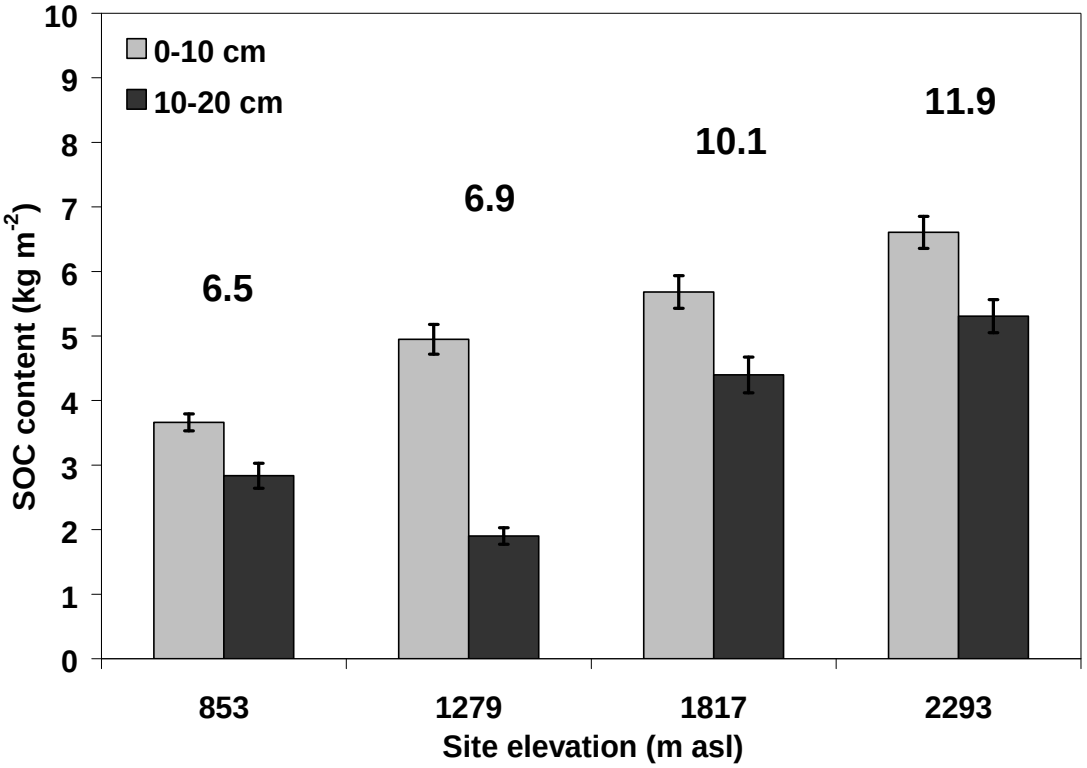


Figure 14. Berguedà: SOC contents across the limestone grassland elevation gradient. Error bars indicate ï1 SE of the mean from 8 replicates. Numbers above bars indicate site total SOC contents (0-20 cm).

Labile C (fPOM C + oPOM C) proportions in the Furka pass siliceous alpine soils were three times larger than those indicated in the Berguedà limestone temperate to alpine soils (Figure 15).

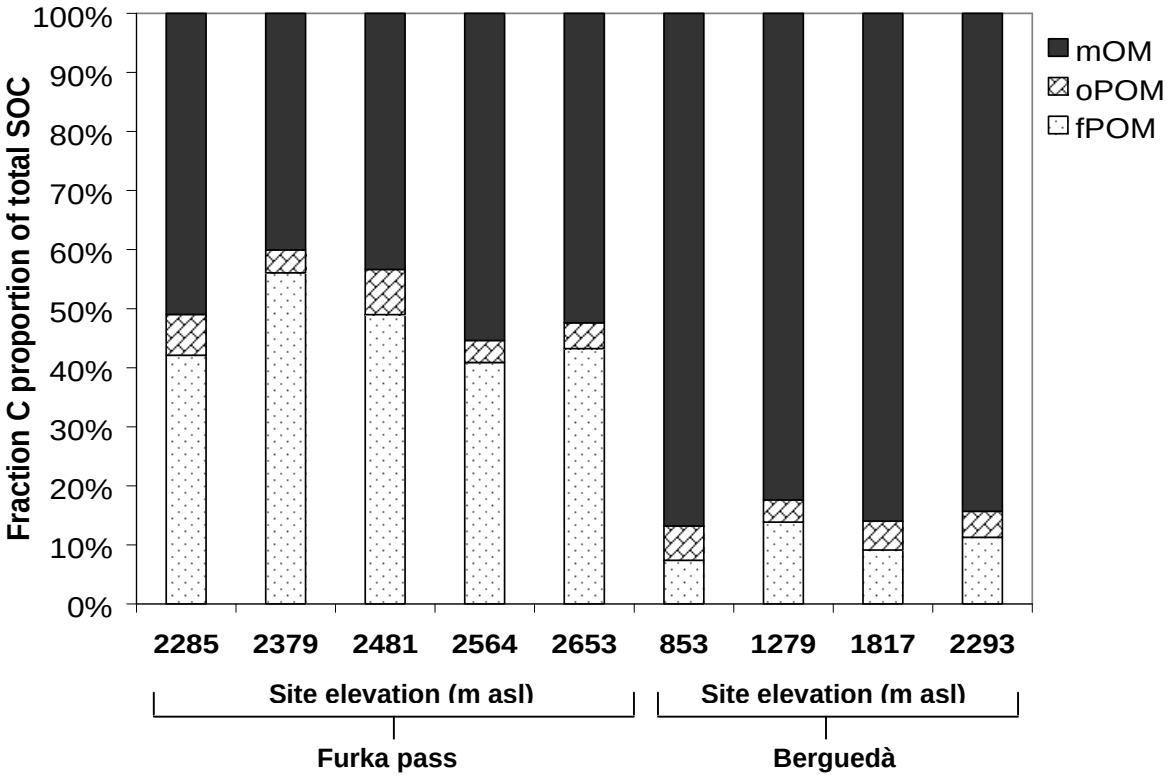


Figure 15. Furka pass and Berguedà: C distribution in soil fractions, from 20 cm soil cores, across silicate and limestone soil grassland elevations gradients.

fPOM C proportions ranged from 40.9-56.1 % in the Furka pass soils compared to 7.4-13.9 % in the Berguedà limestone soils. Proportions of oPOM C were similar in soils from both gradients, ranging from 3.7-7.7 % in siliceous soils and 3.8-5.8 % in the limestone soils.

While oPOM C proportions of total SOC are similar in both soils, the relative proportion of oPOM comprising the labile C (total POM C) is greater in the limestone soils. Relative proportions of labile C comprised by the oPOM fraction ranged from 21.3-44.2 % in the limestone soils compared to 6.5-13.5 % in the siliceous soils. Relative oPOM C proportions of labile POM C from compiled data from both gradients indicated a significant relationship (*r* = 0.87; p = <0.01) with soil clay concentration (Figure 16).

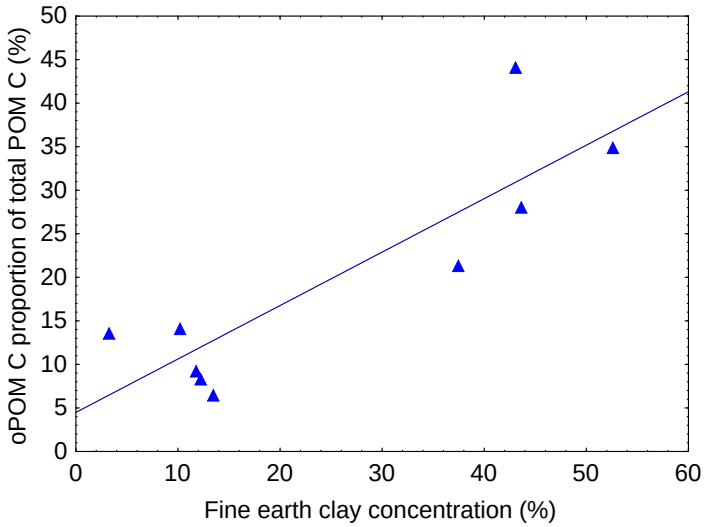


Figure 16. Furka pass and Berguedà: Relationship between oPOM proportion of total POM C and soil clay concentration from limestone and silicate bedrock grassland elevation gradients.

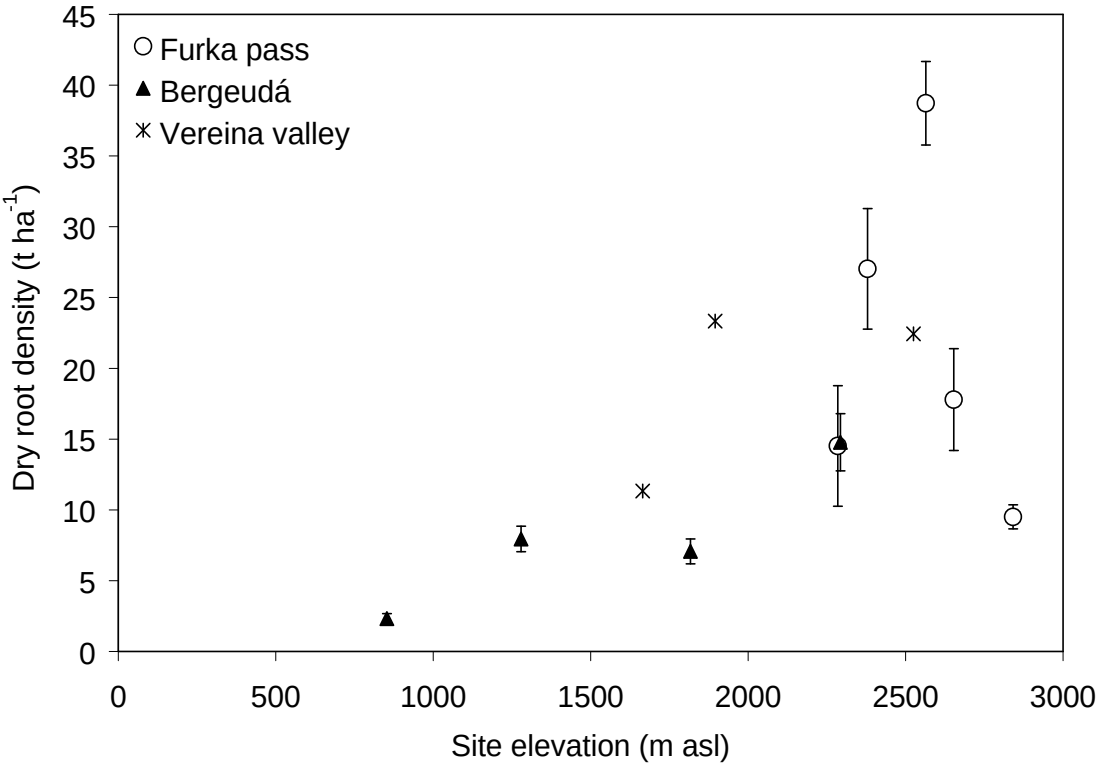


Figure 17. Root densities (0-20 cm) from all gradients plotted against site elevation. Vereina valley values were taken from *Hitz et al. 2001. J. Plant Nutr. Soil Sci. 164: 389-397.*

12.4 Root trends and stone volumes

Across the Swiss siliceous alpine gradient, total root/litter densities (0-30 cm) varied between 10.3 and 42.0 t ha^{-1} dry matter across sites (Table 6), and, as with SOC contents, were higher at 2379 and 2564 m than at the other sites. Stone volume increased, while root/litter densities decreased, with soil depth. Regression analysis revealed a significant linear relationship between site SOC content with both root/litter dry matter density *(r* = 0.93, *p* = 0.02, *n* = 5) and stone volume *(r* = -0.98, *p* = <0.01, *n* = 5). Effect of soil depth was highly significant (*p* < 0.01) across all sites for soil C and N concentration, root densities, proportions of labile C, and bulk densities, but not for soil C stocks.

Across the larger Pyrenean limestone gradient, root dry matter densities were significantly higher (*p* < 0.05) in the upper 10 cm than in the lower at all sites. Total root dry matter densities (0-20 cm) ranged from 2.3 - 15 t ha^{-1} and showed a general increase with elevation across all 4 sites, although this increase was not significant *(r* = 0.91; *p* > 0.05). Root densities were significantly related to soil C stocks *(r* = 0.65, *p* < 0.05, *n* = 32) in 0-20 cm. Stone volume was higher at the two lower elevation sites however; there was no significant trend with elevation (*p* > 0.05).

Root density values (0-20 cm) from the Furka pass and Berguedà gradient were compiled with published data from the Vereina valley gradient and plotted against site elevation (Figure 17). Highest root densities (0-20 cm) were found in at the middle elevation site of the Furka pass gradient while the lowest were found in the Berguedà temperate sites. The Furka pass alpine grassland root densities did not indicate any trend with elevation. However, the Vereina valley and Berguedà grassland sites

indicated a general increase with elevation, although this trend was not significant across either gradient ($p > 0.05$).

This general trend of increase in root density with elevation across the Berguedà limestone gradient was also indicated in the root MRTs from this gradient. Root MRTs increased from 1 year at the lowest elevation site to 8 years at the highest elevation site (Figure 18), however, unlike with the root densities, this increase with elevation was significant ($r = 0.96$; $p < 0.05$).

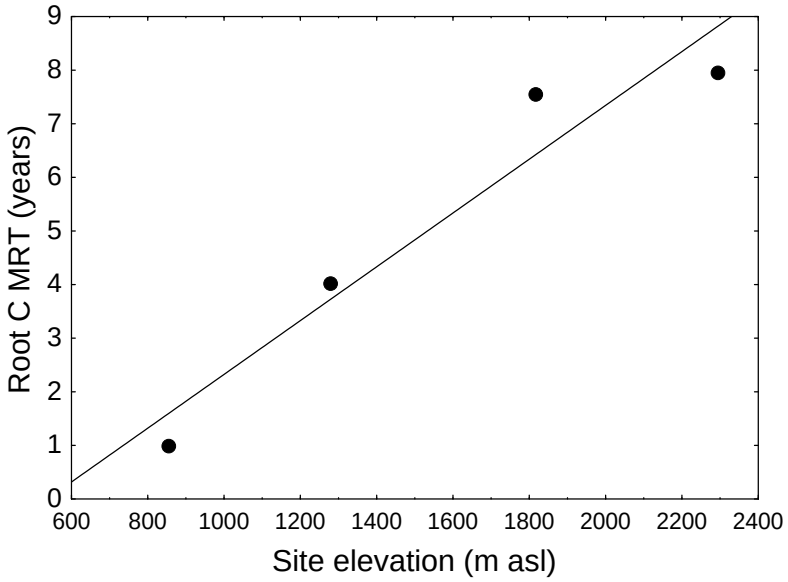


Figure 18. Berguedà: Relationship between root C MRT against site elevation across the limestone grassland gradient.

Table 6. Furka pass and Berguedà: Mean root and stone densities at each sampling site and soil depth.

Site location	Site elevation (m asl)	Soil depth (cm)	Stone volume (%)	SE	Root dry matter (t ha-1)	SE
Furka pass (Swiss Alps)	2285	0-5	5.6	1.3	7.8	1.4
		5-10	11.1	1.0	2.7	0.5
		10-20	17.0	1.2	4.0	2.7
		20-30	16.2	2.9	0.5	0.1
		0-30	*12.5*	1.2	*15.0*	4.3
	2379	0-5	0.4	0.1	18.1	4.0
		5-10	3.3	1.0	6.0	2.1
		10-20	8.0	2.2	3.0	0.3
		20-30	13.4	4.4	1.0	0.2
		0-30	*6.3*	1.3	*28.0*	4.4
	2481	0-5	6.8	1.3	6.0	0.7
		5-10	11.9	2.2	1.7	0.3
		10-20	12.5	1.5	1.9	0.4
		20-30	14.0	1.6	0.8	0.1
		0-30	*11.3*	0.8	*10.3*	0.9
	2564	0-5	1.2	0.4	29.0	1.9
		5-10	4.1	1.2	6.6	1.4
		10-20	6.9	0.7	3.2	0.7
		20-30	12.2	1.3	3.3	0.2
		0-30	*6.1*	0.5	*42.0*	2.9
	2653	0-5	4.1	0.7	14.1	3.1
		5-10	7.8	1.8	3.0	1.3
		10-20	12.4	1.9	0.7	0.2
		20-30	14.4	2.0	0.5	0.1
		0-30	*9.7*	1.1	*18.3*	3.6
Berguedà (Spanish Pyrenees)	853	0-10	*6.8*	1.4	*2.2*	*0.4*
		10-20	*6.1*	1.2	*0.2*	*0.1*
		0-20	*6.5*	*1.2*	*2.3*	*0.4*
	1279	0-10	*7.0*	1.4	*7.5*	*0.9*
		10-20	*20.0*	3.8	*0.4*	*0.1*
		0-20	*13.0*	*2.1*	*7.9*	*0.9*
	1817	0-10	*0.1*	0.1	*6.2*	*0.6*
		10-20	*2.3*	0.8	*0.8*	*0.4*
		0-20	*1.2*	*0.4*	*7.1*	*0.9*
	2293	0-10	*0.0*	0.0	*14.0*	*2.0*
		10-20	*0.9*	0.9	*0.7*	*0.1*
		0-20	*0.5*	*0.5*	*14.7*	*2.0*

(SE) indicate 1 SE of the mean from 6 replicates at Furka pass and 8 at Berguedà

12.5 Labile C trends with elevation

Across the small Swiss siliceous alpine gradient, labile C for 0-20 cm varied in the range of 39.6-57.6 %. Compilation of these values with previous data obtained for lower elevation grassland soils (Leifeld *et al*., 2009; Zimmermann *et al*., 2007) showed an increase in labile C relative to elevation up to 57.6 % at 2379 m, followed by a trend towards decreasing values across the highest three sites sampled here (Figure 19). From the soil profile, the decline in labile C % was not evident in the top 5 cm but occurred in the 5 and 20 cm depth sections.

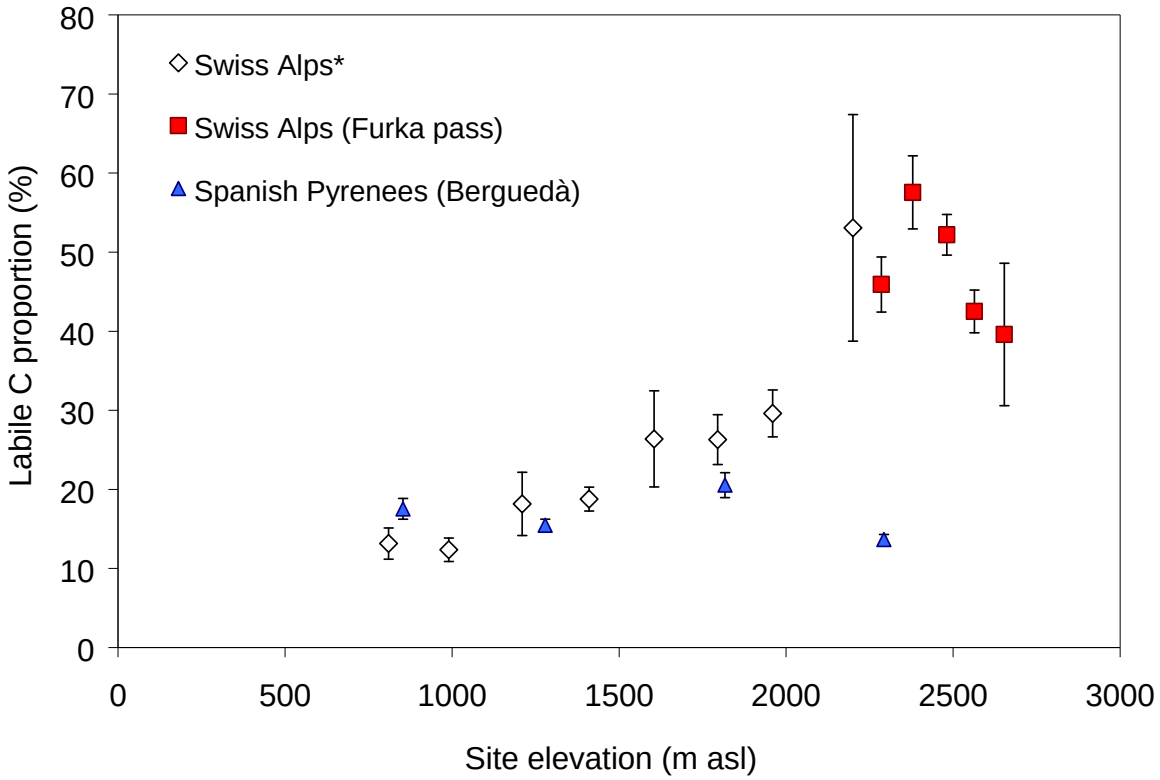


Figure 19. Furka pass sites + lower elevation siliceous grassland sites: Trend in labile C proportion (%) in 20 cm deep soil cores with elevation. Additional data (diamonds) taken from Zimmerman *et al*., 2007 and Leifeld *et al*., 2009. Error bars indicate 1 SE of the

Across the larger Pyrenean limestone gradient, site labile C (POM C) proportions (0-20 cm) ranged from 13.2-17.6 % along the elevation gradient but did not indicate a significant trend with elevation (r = 0.21; $p > 0.05$). Labile C at 0-10 cm depths also indicated no significant trend with elevation (r = 0.64; $p > 0.05$) and although labile C at 10-20 cm indicated a general decrease in proportion with elevation, this decrease was not significant (r = -0.87; $p > 0.05$). Within the labile C fraction at each site, the majority of C was contained within the fPOM portion; site POM C proportions (0-20 cm) of fPOM ranged from 7.4-13.9 % while oPOM ranged from 3.8-5.8 %. Both fPOM and oPOM proportions were larger in upper depths (0-10 cm) with average C proportions across the gradient of 15.5 and 6.5 % respectively, compared with 6.5 and 3.0 % in lower depths (10-20 cm). Labile C proportions and each individual POM C proportion (fPOM and oPOM) decreased significantly ($p < 0.05$) with soil depth.

The data tentatively indicate that preferential accumulation of labile C (in the form of POM) with elevation in grasslands may be specific to siliceous soil. In consistence with previous studies, root densities and MRTs were found to increase significantly with

elevation across this limestone grassland gradient. This pattern seems thus independent of geology.

12.6 Soil fraction degree of transformation

12.6.1 Furka pass

12.6.1.1 C/N ratios

Independent of site, the trend towards decreasing C/N ratios indicated an increase in the degree of microbial transformation from root/litter→fPOM→oPOM→mOM (Figure 20). Root/litter material, fPOM and oPOM all displayed increasing C/N ratios with soil depth, whereas mOM with the lowest C/N ratio did not vary with depth. The depths effect on C/N ratios was highly significant ($p < 0.01$) for fine earth C/N, root/litter, fPOM, and oPOM. Across sites and separated by depth, the difference in C/N ratios between root/litter; fPOM; oPOM, and mOM was highly significant ($p < 0.01$) for all fractions but fPOM and oPOM 0-5 cm.

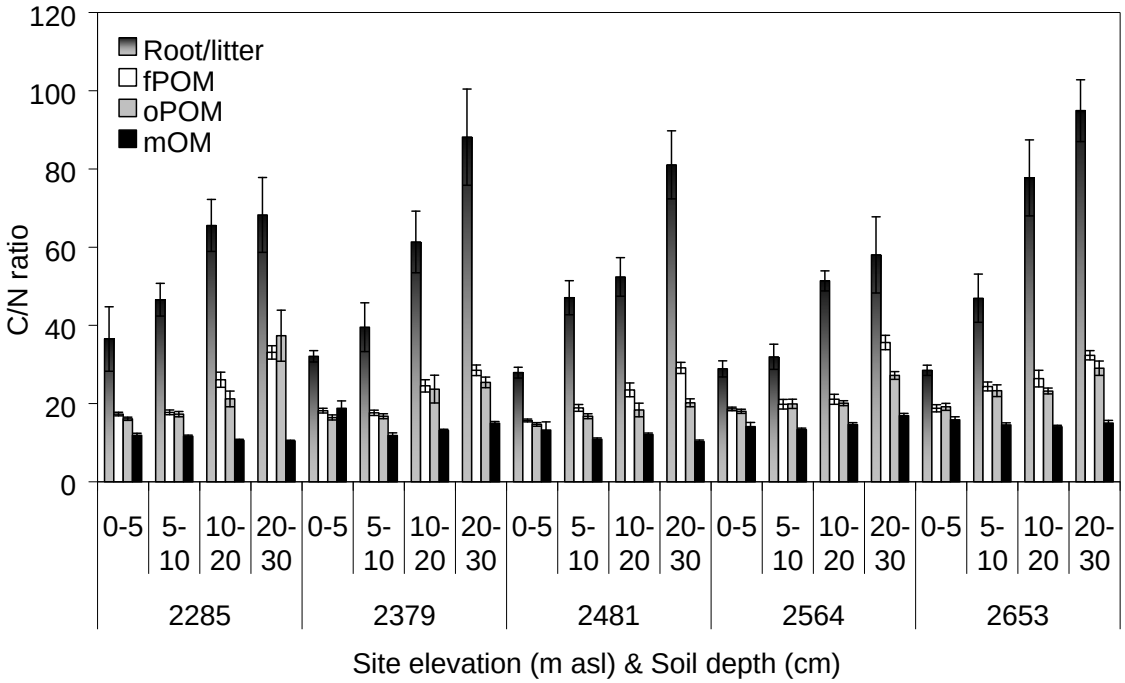


Figure 20. Furka pass: C/N ratio trends of root/litter, fPOM, oPOM and mOM between fractions and with depth at all sites of the siliceous alpine grassland gradient. Error bars indicate 1 SE of the mean from 6 replicates.

12.6.1.2 Chemical groups by NMR analysis

Chemical functional groups measured by NMR spectra in selected samples from the 5-10 cm layer confirmed the varying degree of transformation with a decrease in O-Alkyl-C and an increase in Alkyl-C from root/litter→fPOM→oPOM→bulk soil. The corresponding data summarized in Table 7 show the related increase in Alkyl-C/O-Alkyl-C ratios, which reflected the progressive degree of transformation.

Table 7. Furka pass: Chemical group concentrations and Alkyl-C/O-Alkyl-C for soil fractions, fine earth and root/litter selected from the two uppermost elevation sites of the siliceous alpine grassland gradient.

Site elevation (m asl)	Fraction	Soil depth (cm)	O-Alkyl-C (%)	Alkyl-C (%)	Alkyl-C/ O-Alkyl-C ratio
2653	fPOM	5-10	61.6	21.0	0.34
	oPOM	5-10	57.6	28.9	0.50
	fine earth	5-10	54.0	31.3	0.58
2564	root/litter	0-5	66.2	15.1	0.23
		5-10	66.6	14.7	0.22
		10-20	N.A.	N.A.	N.A.
		20-30	N.A.	N.A.	N.A.
	fPOM	0-5	59.2	25.3	0.43
		10-20	60.7	19.5	0.32

N.A. = not analysed

Additionally, in agreement with C/N ratios given in Figure 20, Alkyl-C/O-Alkyl-C ratios from a single site (2564 m asl) revealed the decrease in the degree of transformation of fPOM with increasing soil depth. Sample values for the O-Alkyl-C % region (60-90 ppm) indicated a negative significant correlation to MRTs (r = -0.95; p = <0.001, n = 8) (Figure 21).

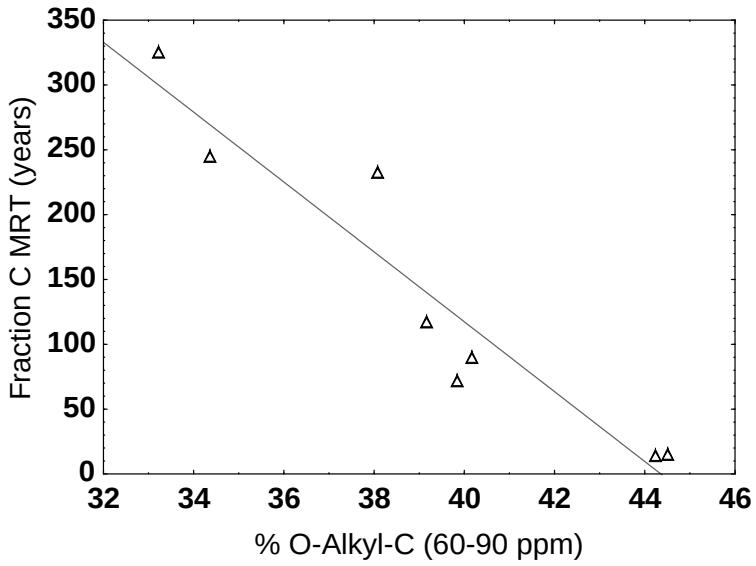


Figure 21. Furka pass: Linear correlation between fraction C MRT and chemical composition (O-Alkyl-C %) in siliceous alpine grassland soil fractions measured by NMR.

12.6.2 Berguedà

Individual C/N ratios decreased from root→fPOM→oPOM→mOM at the lower three elevation sites at both depths (Figure 22); however this trend was not consistent at site

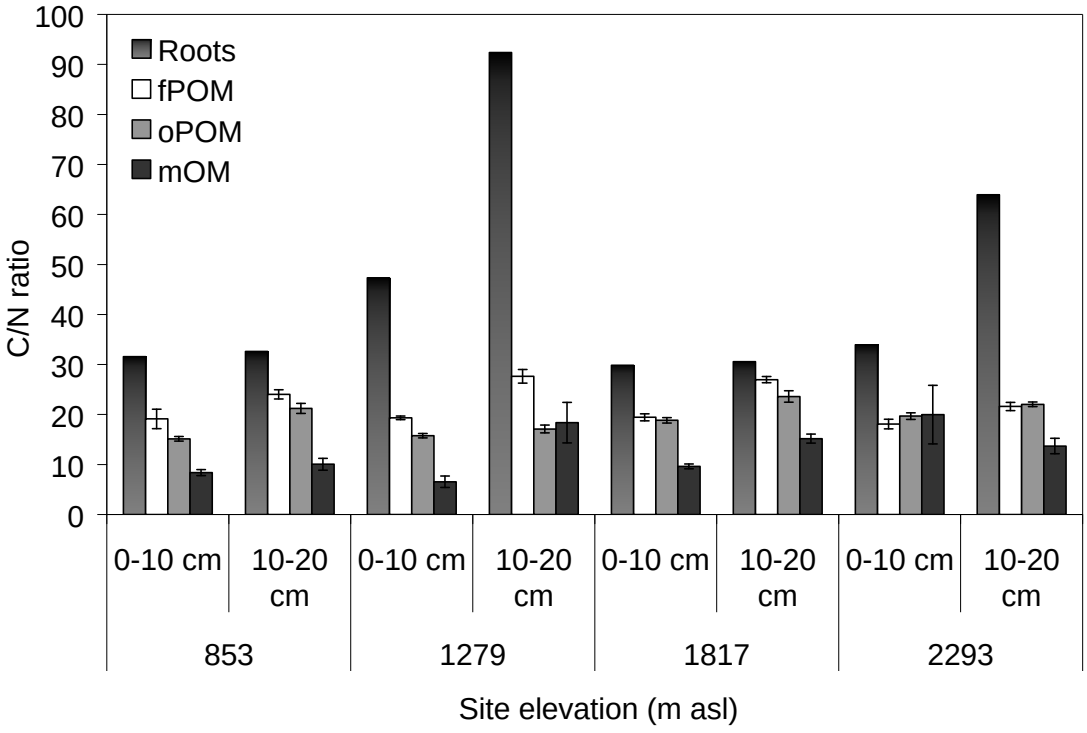


Figure 22. Berguedà: C/N ratio trends of root, fPOM, oPOM and mOM between soil fractions and between depths at all sites of the limestone grassland gradient. Error bars indicate ï1 SE of the mean and were not possible for root samples as individual composite samples (from 8 replicates) were measured.

2293 m asl. Root, fPOM and oPOM C/N ratios increased with soil depth at all sites while mOM C/N ratios only increased with depth at two sites (1279 and 1817 m asl). Site fine earth C/N ratios ranged from 9.6–11.7 and increased non-significantly with elevation *(r* = 0.93; *p* > 0.05). Site root C/N ratios, which ranged from 29.9–48.7, showed no trend with elevation *(r* = 0.19; *p* = > 0.05). C/N ratios of labile fractions were not related to elevation with the exception of oPOM in the 0-10 cm layer, which increased significantly with elevation *(r* = 0.97, *p* < 0.05).

12.7 C turnover

12.7.1 POM fractions

In the Furka pass alpine soils, MRT of C in different fractions was determined in individual samples from the 5-10 cm layer. Comparison between fractions from four of the five sites showed that MRT increased from fPOM→oPOM→mOM (Figure 23), with the corresponding fine earth of the fractions indicating MRTs between those of mOM and oPOM. While fPOM and oPOM MRTs showed no significant trend with elevation, MRT of mOM increased significantly with elevation across four sites *(r* = 0.95; *p* = 0.046; *n* = 4)).

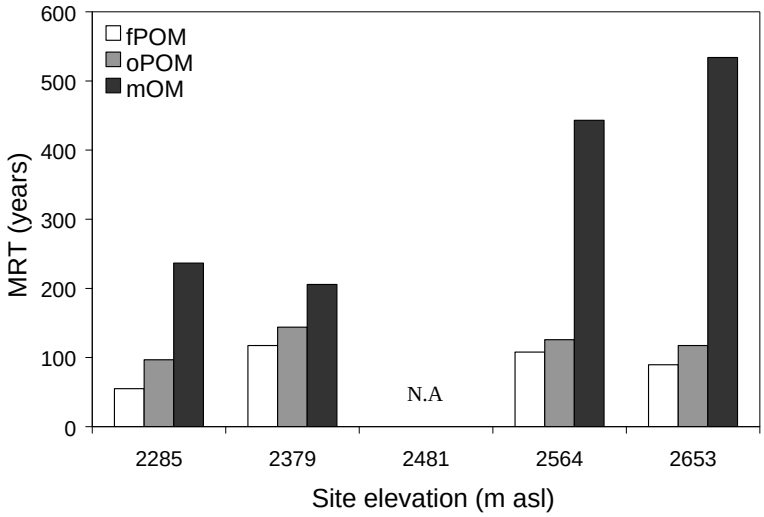


Figure 23. Furka pass: Trend between soil fraction C MRTs, calculated for fractions at 5-10 cm depth, at four sampling sites. Each value represents a single site replicate measured for ^{14}C content.

7.7.2 Fine earth

Fine earth MRT was considerably lower at the middle site and did not increase with elevation across any soil depth (Figure 24) while fine earth MRTs did increase linearly with soil depth at all sites (2285 m: *r* = 0.99; *p* = <0.01; 2379 m: *r* = 0.99; *p* = <0.01; 2481 m: *r* = 0.97; *p* = 0.03; 2564 m: *r* = 0.96; *p* = 0.02; 2653 m: *r* = 0.94; *p* = 0.03; all *n* = 4). Site fine earth MRTs were significantly negatively correlated to site soil pH *(r* = -0.96; *p* = 0.01; *n* = 5) but within the soil profile fine earth MRTs only showed a significant relationship with soil pH in the 0-5 cm depth sections *(r* = -0.96; *p* = <0.01; *n* = 5) and 10-20 cm depth sections *(r* = -0.89; *p* = 0.04; *n* = 5).

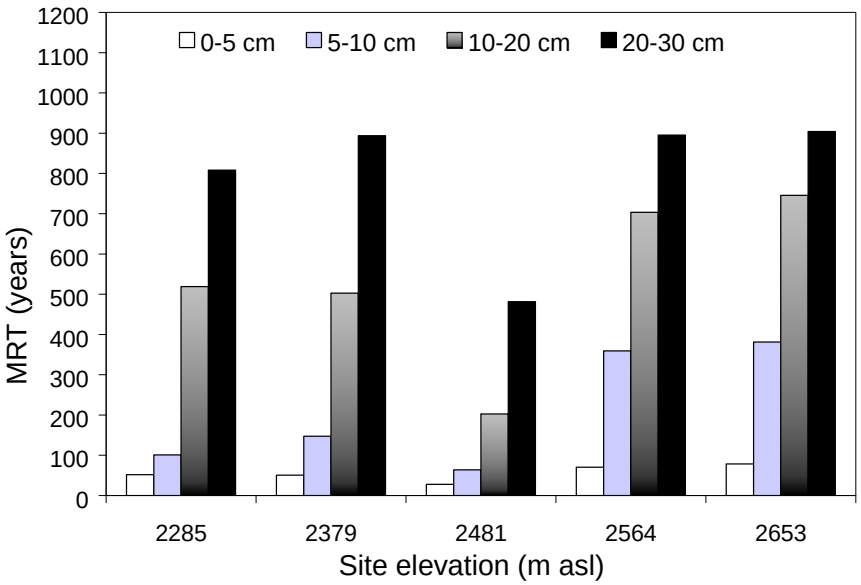


Figure 24. Furka pass: Fine earth carbon MRT with time-lag and recalculation with equation (1), where appropriate, for all depths and sites. pMC values used for calculation were obtained from composite samples measured for ^{14}C content.

12.7.2 Site replicates

MRT of POM for individual site replicates (5-10 cm) varied from 50-76 years along the lower elevation site to 98-126 years along the higher site (Table 8). C input varied by a factor of 3 at the lower and by a factor of 2 at the higher site.

Table 8. Furka pass: Site variability in modern C concentration and residence, content and input of C in fPOM (5-10 cm depth) at two silicate alpine grassland elevations.

Site elevation (m asl)	Rep.	% Modern carbon (pMC)	Carbon MRT (years)	SOC (t ha-1)	Annual C input (t C ha-1)
2285	1	110.9	76	3.2	0.04
	2	114.0	50	5.0	0.10
	3	114.0	50	7.6	0.15
2564	1	107.0	126	4.8	0.04
	2	108.6	102	7.8	0.08
	3	108.9	98	3.0	0.03

12.7.3 MRTs relative to C input and degree of decomposition

MRT of C in 30 cm fine earth samples was lowest at the middle site where annual C input and above ground phytomass were highest though the latter did not differ significantly across sites (Table 9). Conversely, low above ground phytomass and annual C input, in addition to higher phytomass C/N ratios at the uppermost sites (Table 10), were associated with higher MRT. Site pH revealed a significant positive linear relationship with phytomass (r = 0.98; p = 0.003; n = 5). However, calculated

annual inputs were not significantly related to soil pH. Labile C % indicated a positive correlation with total C input (0-30 cm) across all the sites *(r* = 0.96; *p* = 0.012; *n* = 5).

Table 9. Furka pass: Mean fine earth C MRT, annual C input and above ground phytomass at each site. Site values are calculated from 0-30 cm

Site elevation (m asl)	Carbon MRT (years)	Annual C input (t C ha^{-1})	Above ground phytomass (g m^{-2})
2285	105.5	0.52	83.1
2379	115.2	0.85	81.9
2481	55.7	1.01	116.9
2564	185.6	0.55	62.7
2653	168.3	0.40	63.9

Table 10. Furka pass: C/N ratios of plant phytomass and labile organic matter fractions across sites (0-30 cm). Numbers in brackets are 1 SE. Different letters indicate significant differences across sites (Tukeys', p<0.05). N.B. Below ground phytomass is represented by root/litter fraction.

Site elevation (m asl)	Above ground phytomass	Below ground phytomass	fPOM	oPOM
2285	26.2 (1.8) ab	54.2 (4.4) a	17.6 (3.4) ab	25.7 (3.3) a
2379	27.7 (2.0) ab	55.3 (5.9) a	15.9 (0.4) ab	20.6 (1.2) ab
2481	20.7 (1.4) a	50.9 (4.6) a	13.3 (0.3) a	17.7 (0.7) b
2564	23.3 (0.8) ab	42.6 (3.6) a	16.6 (0.4) b	21.3 (0.8) ab
2653	31.7 (4.2) b	62.0 (6.3) a	16.4 (0.4) b	26.6 (3.1) a

Labile material C/N ratios, integrated over 0-30 cm, differed between sites in the above ground phytomass, fPOM and oPOM and were smallest at 2481 m; however this pattern was not indicated with the below ground phytomass (Table 10). Neither above-ground nor below ground phytomass (root/litter) C/N ratios correlated with elevation. However, other root/litter quality parameters, as derived from fibre analysis, were more strongly graded along elevation than above-ground phytomass quality. Root/litter lignin content and the ratio hemicelluloses/lignin were significantly related to elevation (Table 11).

Table 11. Furka pass: Plant quality parameters (g kg^{-1} dry weight) along the elevation gradient. Last row indicates correlation coefficient with elevation; (*) shows significant relationships ($p < 0.05$).

	Hemicelluloses		Cellulose		Lignin		Hemicelluloses/ Lignin	
Site elevation (m asl)	Above ground phytomass	Root/ litter	Above ground phytomass	Root/ litter	Above ground phytomass	Root/ litter	Above ground phytomass	Root/ litter
2285	171.1	200.8	156.3	238.4	105.5	114.4	1.62	1.75
2379	95.2	275.3	244.5	206.0	177.8	134.2	0.54	2.05
2481	105.9	215.9	151.2	251.6	121.2	132.3	0.87	1.63
2564	190.5	179.5	243.2	147.2	121.8	165.0	1.56	1.09
2653	120.5	129.8	125.9	193.5	131.6	176.9	0.92	0.73
correlation	-0.03	-0.70	-0.17	-0.56	-0.03	0.95*	-0.14	-0.89*

12.8 Root C storage and turnover

As with root dry matter, root C contents decreased steeply with depth across all sites, ranged from 0.9–6.0 t ha^{-1} in the upper layers across the sites but indicated no trend with elevation (Table 12). Annual C inputs from root turnover (0-10 cm) did not scale with elevation. They were similar for three out of the four sites, with site 1817 m asl less than half that of the other sites.

Table 12. Berguedà: Root carbon content, modern C concentration, C residence times and C input from 0-10 cm sections at each site of the limestone grassland gradient.

Site elevation (m asl)	Root C (t ha^{-1})	SE	% Modern carbon (pMC)	Carbon MRT (years)	Annual C input (t C ha^{-1})
853	0.9	0.2	102.2	1.0	0.9
1279	3.2	0.4	106.4	4.0	0.8
1817	2.6	0.3	108.7	7.6	0.3
2293	6.0	0.8	109.3	8.0	0.8

Standard errors (SE) indicate 1 SE of the mean from 8 replicates

12.9 Plant species diversity

At Furka pass, a total of 56 plant species (full list given in Appendix 3) were identified across all sites, with 32 of these species only present a single site and 5 species (2 grass and 3 forbs: *Anthoxanthum odoratum, Geum montanum, Helictotrichon versicolor, Leontodon helveticus* and *Potentilla aurea*) present at all sites. Except for the top site, which contained a large proportion of dwarf shrubs and lichens, the predominant functional groups across the alpine grassland elevation were forbs followed by grasses. The relative distribution of each functional group varied greatly between the sites (Figure 25).

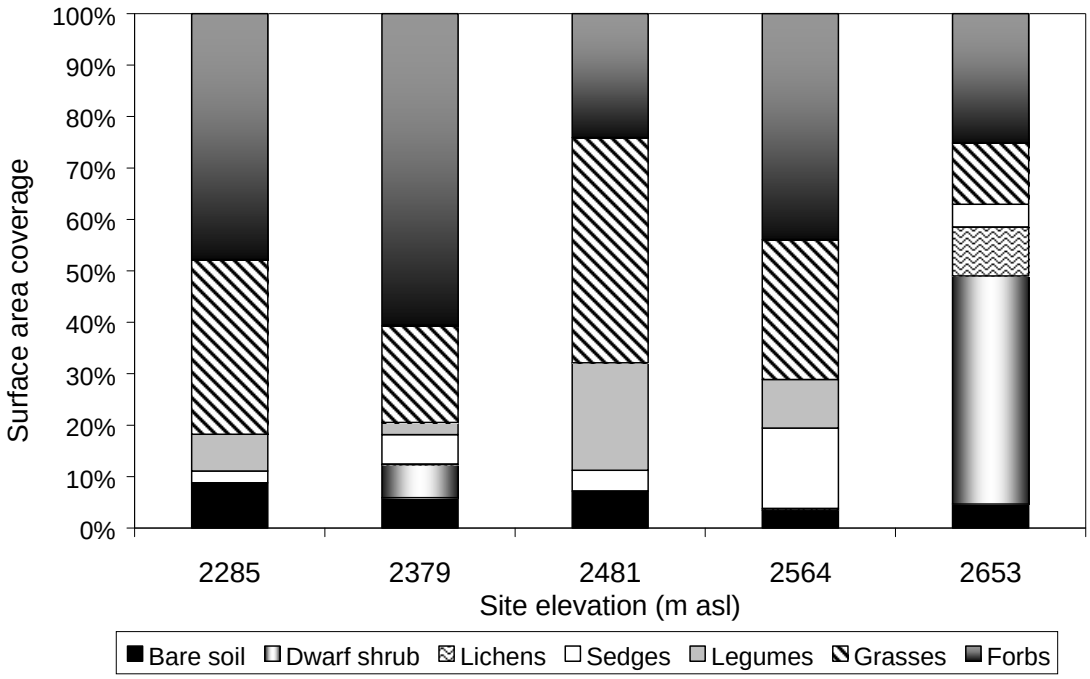


Figure 25. Furka pass: Functional group surface area distribution (%) of vegetation at each elevation site of the siliceous alpine grassland gradient.

At the middle site (2481 m) the fraction of legumes was largest and a preference for higher pH was indicated by the Ellenberg value (Figure 26). Ellenberg values for soil moisture and nutrient availability did not indicate any trend with elevation. Temperature value decreased slightly from 1.7-1.8 at 2564 m and below to 1.5 at 2653 m, thus indicating a small shift in plant community towards a preference for colder temperatures.

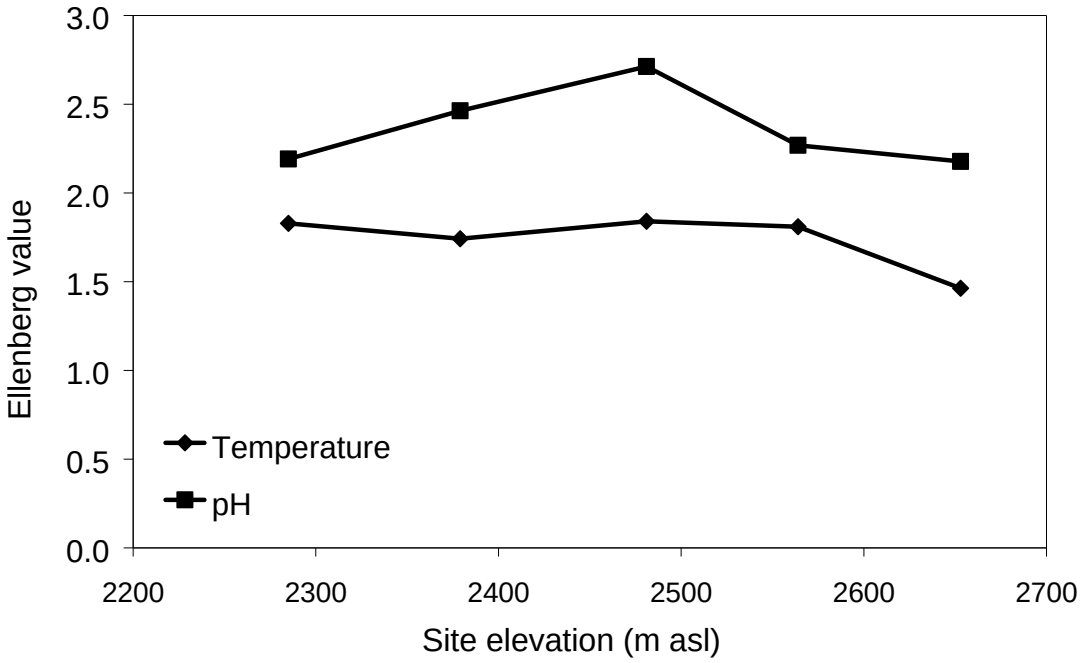


Figure 26. Furka pass: Ecological preference of vegetation indicated by vegetation species across alpine grassland elevation gradient. For temperature an increasing Ellenberg value indicates preference for warmer temperatures while with respect to pH, increasing Ellenberg values indicates preference for higher alkalinity.

12.10 Soil microbial community

12.10.1 Total microbial biomass

TMB was lower in the 10-20 cm than in the 0-10 cm layer at all sites (Figure 27). Soil TMB at the Stutzegg site, Jöri-Vereina cores and Jöri site showed a strong significant decrease with depth.

From the upper to the lower layer, TMB decreased by 65, 79 and 91% at the Stutzegg site, Jöri-Vereina cores and Jöri site, respectively, whereas the reduction at the Vereina site was only 9% (not significant). One-way ANOVA tests indicated a significant difference between sites at 0-10 cm ($p < 0.01$) and at 10-20 cm depth ($p < 0.01$). Further analysis of individual sites revealed that TMB did not vary significantly between the Jöri-Vereina cores and the Jöri or Stutzegg site at either depth. In contrast, TMB varied significantly between the Jöri-Vereina cores and Vereina site at 0-10 cm and 10-20 cm ($p < 0.01$). TMB at the Vereina site also differed significantly from the two other sites at 0-10 cm and with the Jöri site at 10-20 cm ($p < 0.01$).

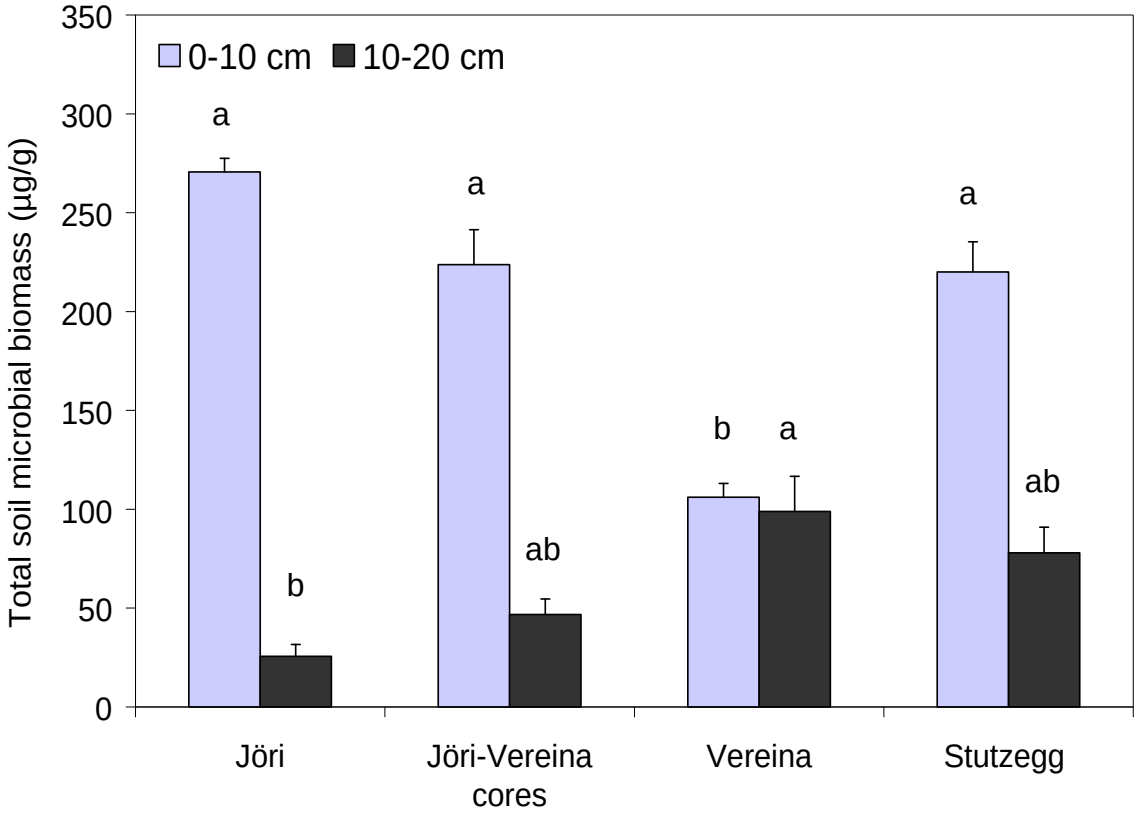


Figure 27. Vereina valley: Total soil biomass (in µg per g of soil) of cores and investigation sites at two depths. Error bars indicate 1 standard error of the mean. For each depth, bars labelled with the same letter are not significantly different at p<0.05.

12.10.2 Individual PLFA indications

Highest contents were found for the following PLFAs: i15:0, 16:1u7c, 16:0, 18:1u9, 18:1u7 and cy19:0 (Appendix 4). Individual PLFA contents were generally lower at the 10-20 cm depth, but, with exception of the Vereina site, differed little between sites. PLFA contents at the Vereina site were consistently much lower than those of the other two sites and than those of the Jöri-Vereina cores. Contents in Jöri-Vereina cores were similar to those at the site of origin (Jöri). Differences in fatty acid composition could be represented by three principal components (PC) that together accounted for 60% of the variability (PC1 31.8%, PC2 18.2%, PC3 10.3%). PC factor 1 indicates differences in fatty acid composition between Jöri and Jöri-Vereina (10-20 cm) and the other sites (Figure 28).

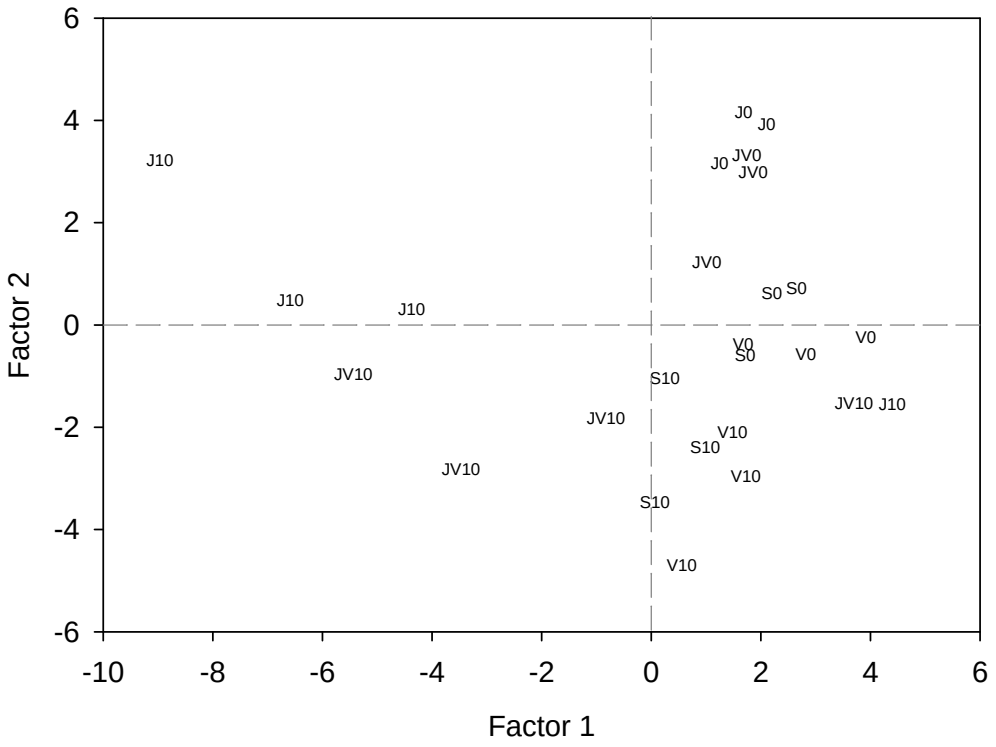


Figure 28. Vereina valley: Scores of first two factors of principal component analysis of individual PLFAs'. The first factor explains 31.8 % and the second factor 18.2 % of the total variance. S, V, JV, and J stands for Stutzegg, Vereina, Jöri-Vereina and Vereina. '0' stands for 0-10 cm, '10' for 10-20 cm soil depth.

PC1 was highly negatively correlated with soil pH *(r* = -0.89, *p* < 0.001) but as deeper layers had consistently higher pH this may indicate an effect of soil depth. Positive loading on factor 1 was mainly driven by cy19:0 whereas negative loadings where mainly attributed to 10Me16:0 and 18:0. Factor 2 had highest positive loadings on 18:2u6,9 and on 10Me17:0 and tended to distinguish sites (separately for 0-10 and 10-20 cm) in the order of Jöri/Jöri-Vereina/(Vereina, Stutzegg). Factor 3 had highest positive loadings on 16:1u5c and 10Me17:0. Factor 3 correlated negatively with % SOC and C/N ratio *(r* = -0.68 and r = -0.66, *p* < 0.01) and is just related to substrate composition.

12.10.3 PLFA ratio indications

In contrast to the absolute amounts, the fraction of total cyFA proportions (cy19:0 + cy17:0) relative to TMB was highest at the Vereina site (Table 13). Among the other soils, the fraction increased from Jöri/Jöri-Vereina cores/Stutzegg in both soil layers. The proportion of EM fungi varied between the sites and was highest at the top elevation and lowest at the Vereina site. Across the three other soils, % EM fungi tended to decrease in both soil layers from the Jöri site/Jöri-Vereina cores/Stutzegg site while % AM fungi increased across these sites in the lower depths, but without significant differences between sites. No clear trend was observed in % AM fungi between sites for the upper layer. Proportions of AM fungi were always higher than those of EM fungi. In the upper layer, the Vereina site had the highest proportion of Gram(+) bacteria. The proportion in the upper layer was less than in the lower layer

where it increased from Jöri site/Jöri-Vereina cores/Stutzegg site, but differences between sites were significant. CyFA/monoenoic precursors ratios decreased from the Jöri site/Jöri-Vereina cores/Stutzegg site in both soil depths and increased with soil depth in the cores, but not in soil from the Vereina site.

Table 13. Vereina valley: Mean microbial biomarker in relation to total microbial biomass (%) and indicator proportions of soil from cores and investigation sites at two depths. Numbers in columns followed by the same letter are not significantly different at $p < 0.05$.

Site	cyclopropane (cy)FA's (%)		EM fungi (%)		AM fungi (%)		Gram (+) bacteria (%)		cyFA/monoenoic precursors ratio	
	0-10	10-20	0-10	10-20	0-10	10-20	0-10	10-20	0-10	10-20
Jöri	10.4 b	9.8 b	8.9 a	9.3 a	11.7 b	9.2 b	14.8 b	18.0 a	1.5 a	2.9 a
Jöri-Vereina cores	11.9 b	10.1 b	4.5 b	2.1 a	11.3 b	11.8 a	17.9 a	22.7 a	1.4 a	2.0 b
Vereina	16.3 a	13.8 a	1.7 b	2.2 a	14.4 a	13.5 a	19.7 a	23.5 a	1.3 ab	1.0 c
Stutzegg	12.4 b	12.2 ab	3.8 b	1.4 a	16.3 a	14.9 a	17.6 a	23.5 a	1.1 b	1.4 bc

12.10.4 PLFA chemical group indications

Mono-unsaturated fatty acids (mUS) made up the highest proportion of TMB in both soil layers at all sites while polyunsaturated fatty acids (pUS) represented the lowest proportion of TMB, except at Jöri (Figure 29). The highest site (Jöri) differed significantly ($p < 0.05$) from the lower sites (Vereina and Stutzegg) and from the Jöri-Vereina cores in the tbS fraction and pUS fraction of the upper layer and in the pUS fraction of the lower layer.

The fractional proportion of the tbS was highly variable between the lower depth replicates of the Jöri site and therefore this site was not significantly different ($p > 0.05$) from the other sites despite the site mean indicating a lower value. In the 10-20 cm layer, the differences between sites tended to be more pronounced. Fractional proportions of scS and mUS did not differ significantly between the sites or cores in the upper layers while significant variation was indicated in the lower layers. In addition, consistent positive or negative trends across the Jöri/Jöri-Vereina cores/Stutzegg sites were observed for all fractions in the lower layers while this trend was only indicated in the mUS and pUS fractions of the upper layers. The data for the local Vereina soil differed markedly from these trends, and differed significantly from the translocated cores in the mbS and pUS fractions of the upper layers and from the mbS, scS and mUS fractions of the lower layers.

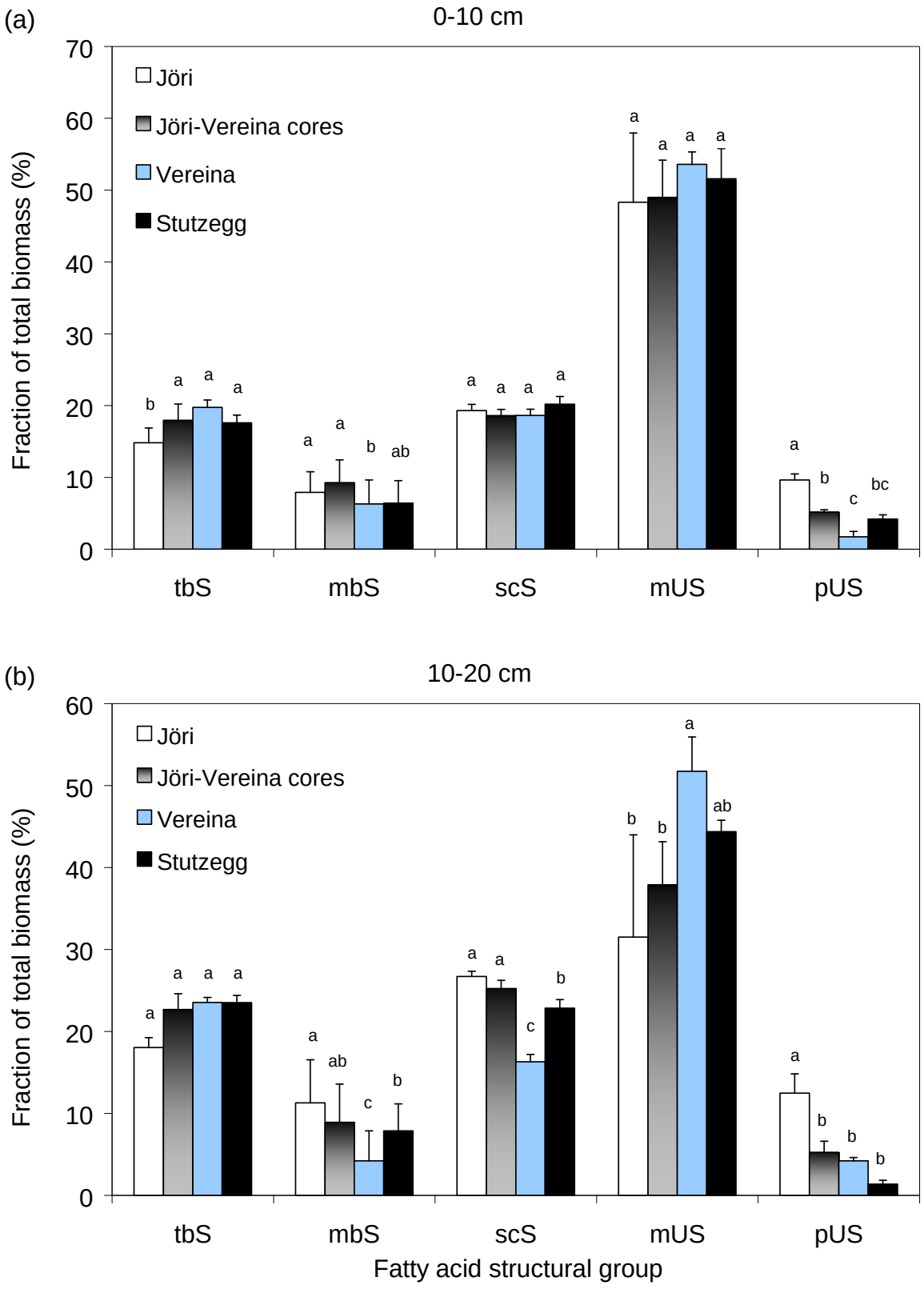


Figure 29. Vereina valley: Chemical group (%) in cores and translocation sites at (a) 0-10 cm and (b) 10-20 cm for the following groups: terminal methyl-branched saturated (tbS), mid-chained methyl-branched saturated (mbS), straight chain saturated (scS), mono-unsaturated (mUS) and polyunsaturated (pUS). Error bars indicate 1 SE of the mean. Bars labelled with the same letter are not significantly different at $p < 0.05$.

13 Discussion

13.1 SOC contents and trends

Soil from the central Swiss Alps contains SOC contents comparable to those reported for lower elevations, but the percentage of root/litter material in the top 5 cm and POM contents in the top 10 cm are considerably higher than those reported for temperate and subalpine grasslands in Switzerland (Ammann *et al*., 2009; Leifeld *et al*., 2009*).* While SOC contents across this small elevation siliceous alpine grassland gradient did not indicate any trend with elevation, results indicated that variability of SOC contents correlated to other site factors, such as stone volume and root/litter density in these soils. This correlation between stone volume and SOC content has been reported previously in siliceous grassland soils (Leifeld *et al*, 2005; Leifeld and Fuhrer, 2009) and is likely to be a result of the physical limitation of storage capacity created by stone content, this may also be the case with dense root/litter content.

These Berguedà Pyrenean limestone grassland SOC contents are similar to those reported previously for subalpine-alpine Pyrenean limestone grassland soils. Garcia-Pausas *et al*. (2007) reported SOC contents of 5.9–21.2 kg m^{-2} in their limestone soils, from 7 sites across an elevation gradient of 1845–2560 m asl, measured from sampling depths of up to 0.78 m. Converted to depths of 0.2 m, they observed average stocks of 9-12 kg m^{-2} which is similar to the two uppermost sites of this limestone gradient. While it is notable that sites in the Garcia-Pausas *et al*. paper experienced higher MAP and higher soil acidity than the sites of this study, there was no trend in SOC content with elevation reported in their soils. This was also the case in limestone soils of the Austrian Alps where Djukic *et al*. (2010b) reported similar SOC contents in alpine grasslands of 13 and 26 kg m^{-2} but found no trend in SOC contents along their elevation gradient of 900-1900 m asl. In addition, Schindlbacher *et al*. (2010) found no trend in SOC content along an elevation gradient of 890 to 1556 m asl on limestone in the Austrian Alps. Both studies included different land-use types and management intensities, factors which may superimpose any relationship between elevation and C stock. In contrast, SOC contents increased significantly with elevation across the 4 Pyrenean sampling sites in this study, despite decreasing bulk densities. This may indicate that soil C stocks increase with elevation but that such a pattern can only be identified for soils of matching parent material, land-use and management, which is sampled down to the same depth.

13.2 Labile C proportion

13.2.1 Trends with elevation

A higher labile C % compared to values found at elevations below 2000 m confirms the high abundance of labile C in the top soil layer, whereas below 10 cm, labile C % is only slightly higher (10-20 cm) or very similar (20-30 cm) to that at lower sites (Leifeld *et al*., 2009; Zimmermann *et al*., 2007). The labile C proportion (0-20 cm) found in all of the siliceous soil alpine sites in this study were indeed higher than those reported in siliceous soils of lower elevation/ temperate sites (Figure 19) and is in line with data from the few studies carried out on drier alpine tundra soils, e.g. in the Tibet mountains where similar total C contents were found at high and low elevations, but where the labile C content in the top soil layers was considerably higher at the higher sites (Wang *et al*., 2005; Wang *et al*., 2008). However, the data found here reveal maximum labile C of around 58 % (0-20 cm) at 2379 m and a decline with a further increase in elevation due to declining contents mainly below 5 cm. As labile C proportions across this alpine grassland gradient did not indicate any direct correlation with any of the other soil

characteristics measured, it is difficult to theorise a reason which may explain this pattern. However, it may be that labile C accumulation in very high elevation grassland soils is limited by the increased proportions of mineral-associated material observed in these soils. Alternatively, or additionally, it may be a result of the variation in the decomposition of plant species, for example dwarf shrubs present at the top elevation site are less readily degradable than grass species (Springob and Kirchmann, 2002).

Labile C proportions in the Pyrenean limestone grassland soils were on average 15 % (0-20 cm) across the elevation gradient and, in contrast to the significant increase with elevation indicated in their SOC contents, did not indicate any trend with elevation. The labile C proportions in these soils were less than half those found in the Swiss alpine siliceous soils, not only at the lower grassland sites, but also at the only alpine site included in the limestone elevation gradient, where the proportion of labile C was still only 16 % above 2000 m. The greater proportion of labile C in the siliceous alpine soils is a result of the considerably larger fPOM C proportions in these soils compared to the limestone soils (Figure 15); oPOM C proportions of total SOC were similar in soils from both gradients. However, oPOM C proportions relative to total POM C content indicated that a larger proportion of POM material in the limestone soils is aggregate protected than in the siliceous soils. Comparison of relative oPOM proportions indicated a significant relationship with clay content, which may increase aggregate formation of POM material in these limestone soils. Correspondingly, lower proportions of oPOM with lower clay contents have also been reported previously in Cambisol soils (Kölbl and Kögel-Knabner, 2004).

13.2.2 Alpine soils as CO_2 hotspots

While the relatively low labile C proportion found at the limestone alpine site did not indicate a large proportion of readily degradable material, the labile C proportion found in all of these siliceous soil alpine sites indicates that there is, comparatively speaking, a large amount of readily degradable C in these soils. Due to this readily available source of C, it is therefore possible that these siliceous alpine soils could be C hotspots in the event of climate warming. This scenario is based on the premise that accumulation of labile C is favoured by low temperatures and that rising air temperatures could lead to an increase in decomposition, consequently releasing CO_2 from these soils into the atmosphere. However, it is unlikely that temperature alone is the driving variable in the accumulation of labile C observed with elevation in these siliceous grassland soils. While there was no trend observed with soil temperature across the elevation gradient and as only a small air temperature of ~0.6 °C between each site of this alpine elevation gradient would be expected, it is difficult to consider what role temperature alone may play with respect to variation in labile C proportion between the sites of this small elevation gradient. However, even within this small elevation gradient trends in litter quality and a strong effect of soil pH were observed and this highlights the importance of factors beyond temperature in the influence of soil C storage and distribution.

Compilation of the labile C proportions from alpine siliceous grassland soils with data from temperate sites allows for consideration of variation across a larger elevation gradient (and therefore MAT gradient). It is notable that when labile C proportions of siliceous grassland soils are plotted against MAT (Appendix 5), a significant trend of decreasing proportion of labile C with increasing MAT is observed. However, while this relationship does suggest that temperature may play a role in labile C accumulation of siliceous grassland soils, as discussed above, it is also likely that other soil processes play influential roles in this trend.

13.2.3 Comparison of elevation gradient studies

The increase with elevation in SOC contents but no trend with elevation in the labile C proportions in these limestone Pyrenean grassland soils is the reverse pattern of that observed in the Swiss siliceous grassland elevation gradient of this study and that of another Swiss siliceous grassland elevation gradient of similar elevation 810-2200 m asl (Leifeld *et al*., 2009). These results are also in contrast to a study in the alpine tundra soils of Tibet (Wang *et al*. 2005) where SOM content of litter layers increased with elevation from 30 % at 1700 m asl to >50 at higher elevations up to 3900 m asl on siliceous soils under varying vegetation. Although these study locations differ in many respects it is notable that studies where significant increases in the labile fraction with elevation were observed were all under siliceous bedrock, with correspondingly lower pH values, than the Pyrenean limestone grassland sites of this study.

13.3 POM stability and degree of decomposition patterns

13.3.1 Furkapass

The data in this study support the view of soil as a hierarchical system of aggregates where intra-aggregate material is protected, but already more transformed (Tisdal and Oades, 1982; Six *et al*., 2004). In these siliceous grassland alpine soils, the trend towards increasing degree of transformation from root/litter → fPOM → oPOM → mOM both in C/N ratios and in alkyl-C/O-alkyl-C ratios is consistent with findings from temperate soils (Golchin *et al*., 1994a,b; Baisden *et al*., 2002), and is in line with systematic differences in composition between fPOM and oPOM reported for soils in climatically different regions (Golchin, 1994a; Kölbl and Kögel-Knabner, 2004). In subtropical soils, Golchin *et al*. (1994b) reported mean alkyl-C/O-alkyl-C ratios of 0.43 (fPOM) and 0.92 (oPOM). Ratios reported for POM in temperate soils indicated POM to be less decomposed and to range from 0.20-0.32 in fPOM, 0.28-0.36 in oPOM (Kölbl and Kögel-Knabner, 2004), 0.37 in mOM and 0.44-0.50 in fine earth (Helfrich *et al*., 2006).

In these alpine soils, alkyl-C/O-alkyl-C ratios of the root/litter fraction were similar to those reported previously for agricultural crops including grass-clover roots (Leifeld and Kögel-Knabner, 2005). Alkyl-C/O-alkyl-C ratios of fPOM in layers below 10 cm depth were also similar to those reported in temperate soils but ratios in the 0-10 cm layers pointed to a much higher degree of transformation. In addition, oPOM was more transformed than those from temperate soils. Together this indicates that, in the topsoil, long residence times of POM correspond with a higher degree of transformation, in comparison to temperate soils. On the other hand, POM in deeper layers is only slightly transformed which, together with long MRTs, indicate alpine subsoils to be biologically quite inactive. This supports the notion that, in alpine soils, accumulation of both POM fractions is due to their long MRT, with variability in labile C % at the field scale being related to variability in residue inputs.

13.3.2 Berguedà

Trends at the Pyrenean limestone grassland gradient of decreasing C/N ratios between soils fractions and increasing C/N ratios with depth were in line with those of the siliceous alpine soils of this study and previously reported mediterranean/subtropical and temperate grassland soils (Golchin *et al*., 1994; Baisden *et al*., 2002).

In both the siliceous and limestone soils, C/N ratios of POM resemble the pattern of roots, underpinning the importance of roots for the formation of POM in grassland soil. C/N ratios of fPOM in the upper 10 cm, ranging from 18.1-19.4 in the limestone soils, were similar to those of the fPOM fraction in the upper 10 cm of the siliceous alpine

soils, which ranged from 17.3-22. However, they were higher than those reported in temperate soils in the mineral-free low-density fraction in the upper 10 cm, which ranged from 14.5-16.5 (Baisden *et al*., 2002). While these sites vary in factors which may be important in determining the decomposition of litter material and consequent distribution of POM fractions within the soil - such as soil texture, pH and climate - it is notable that these trends in C/N ratios between fractions and depths are consistent despite variation in climate and soil type. They thus reflect functional patterns.

13.4 Chemical composition of SOM relative to MRT (Furka pass)

Across the Swiss alpine siliceous grassland soil gradient, root/litter and POM fraction C/N ratios (Figure 20) and fine earth soil MRTs (Figure 24) generally increased with soil depth. In contrast, C/N ratios of mOM, which are generally lower than in other fractions, revealed no consistent trend with soil depth. This increase in C/N ratio of root/litter fraction is reflected in the increase of C/N ratio of both POM fractions with soil depth, thus indicating a decreasing degree of transformation in labile material with soil depth. This finding is corroborated by NMR results which suggest a pronounced difference in the degree of microbial transformation of fPOM between increments in soil depth (Table 7). Restriction of decomposition in deeper layers may therefore be a result of poor litter quality in combination with low macronutrient content; particularly at lower depths where across the five sites, nutrient concentrations in the 10-20 and 20-30 cm layers were only ~10 % of that in the top 5 cm. In combination, high C/N ratio, nutrient limitations, and possibly restricted physical access due to higher bulk densities, may cause the longer MRT of soil C in deeper soil layers. Therefore, potentially labile and little transformed C sources, such as fPOM, may age at deeper layers without being further transformed due to environmental constraints in these alpine soils.

MRT of POM fractions were in the range of 55 to 144 years and thus higher than those estimated for temperate or tropical soils (Hsieh, 2009), but similar to values found for other cold and acidic soils (Schulze *et al*., 2009). Strong soil profile gradients in litter quality and nutrient availability additionally shape the distribution and turnover of POM with depth. Most strikingly, across a variety of fractions and sites 90 % of the variability in MRTs could be explained by the content of O-alkyl-C (mainly polysaccharides) (Figure 21), showing the strong role of litter or SOM quality on C turnover in alpine soils.

13.5 MRT site variation (Furka pass)

Examination of replicate samples from two of the alpine grassland elevation gradient sites revealed considerable spatial variability in SOC storage and MRT of fPOM in 5-10 cm sections (Table 8). At the lower of the two sites, MRT varied by as much as 34 % between replicates, while at the upper site the coefficient of variation was 22 %. This difference in MRTs between replicates at a single sampling site is important for the estimation of MRT by the bomb model as, due to the high costs of radiocarbon measurement, often only very few representative samples are measured and site variability is not considered. However, variation in C input between replicates was greater than variation in MRT. This suggests that litter input may be a more important factor than turnover rates in determining the spatial variation in C stocks at each elevation, however, as a small number of replicates were used, further replicate measurement is necessary to establish if this is a consistent pattern.

13.6 Improvement of MRT estimations

The bomb model assumes a steady state environment but in the soil studied this assumption may not be the case. However, in this study it is considered that no long term trend in input and turnover exists at the selected sites given that they are only extensively grazed. The model is based on a number of calculated atmospheric and fraction curves which indicate an increasing pMC with time until reaching a peak concentration. The curves thus mimic the atmospheric concentration, but are smoothed depending on the fraction's MRT. The overall pMC observed in each fraction curve depends on the rate of atmospheric absorption; hence faster fractions contain a larger pMC than slower fractions. The model can thus indicate more than one possible MRT, particularly in the fast moving fractions where many fraction curves overlap. Knowing the signature of C entering the system may help to solve this issue (Trumbore *et al*., 1997).

The measurement of individual soil fractions from the Swiss alpine siliceous grassland soils revealed increasing age between POM fractions and mOM. This characteristic is an important feature of the soil when considering MRT of fine earth C, particularly at the higher elevation sites where MRT of mOM is over 4 times that of fPOM fractions. The method used for final MRT estimates, referred to as fraction calculated MRTs, allowed for the overall contribution of the soil fractions to the fine earth. The combination of the addition of the time-lag period derived from root dating into the bomb model, and equation recalculation to the fine earth MRTs with respect to fraction contribution, enables a more realistic estimation of the actual fine earth MRTs. This is an improvement over single measurements which would require a homogenous system and not take this characteristic into account. The application of the time-lag period, which accounts for the period of C residence within the plant tissue, may be particularly important in these soils with a large input from roots. In this case, the inclusion of the time-lag period (i.e., the mean age of roots) into the bomb model indicates which values should be disregarded and therefore the only likely possible MRT time was allocated to the pMC value.

13.7 Factors influencing SOM turnover

13.7.1 Temperature

As discussed above, the small variation in soil temperature, observed between the sites of the Furka pass elevation gradient, indicates that soil temperature cannot explain SOM trends. From repeated visual inspections of the site it seems that due to a longer lasting snow cover at lower sites in the study region soil temperature does not reflect the existing gradient in air temperature. A difference of 1.5 – 2 °C air temperature can be estimated from the difference in elevation between the highest and lowest sampling sites and Ellenberg values confirm a shift in plant community towards preference for colder temperatures (Figure 26). Therefore, changes in C distribution attributed to temperature could occur through shifts in plant community rather than through direct effects on the soil.

13.7.2 Soil acidity

In these siliceous grassland alpine soils, MRT of fine earth C at the middle site (2481 m) is much shorter than expected from the trend across the elevation gradient (Table 9). The soil at this site is characterized by the highest soil pH, particularly in the upper soil layers, highest productivity and total annual C input. The MRT of this middle site, to a depth of 20 cm, was 46.5 years which is similar to those reported at lower elevation siliceous grassland soils, which ranged from 31-47 years between an elevation gradient

of 900-1795 m asl (Leifeld *et al*., 2009). However, the remaining high elevation sites indicated longer MRTs, ranging from 88-153 years, and therefore slow turnover of SOM in these soils,

Soil acidity could be a major driver for the plant-soil system. Based on a previous study on pH effects on C turnover in higher elevation grasslands (Leifeld *et al*., 2008), it is estimated that in the range of pH 4-5, a decrease of ca. 0.5 – 1 units between the higher and lower sites relative to the less acidic middle site, should induce an increase in MRT by a factor of 1.6 to 2.3. However, averaged over the four soil depths, an even stronger increase in MRT of 1.8 to 3.9 times was observed (Figure 24; Table 9) indicating that additional factors must come into play. Across sites, the increase in mean MRT relative to the middle site was highest at the highest elevation, which also differed in vegetation community. This suggests that soil C turnover in alpine grassland is not only directly influenced by temperature and soil acidity but may depend on the vegetation community, in which case plant species variation, plant productivity and the consequent variation in quantity and quality litter input could all be relevant.

In these siliceous alpine soils, slightly less acidic soil corresponded to higher plant productivity and, in turn, to larger inputs of litter with lower MRT; higher productivity was compensated for by faster turnover leading to similar SOC contents as in alpine soils of lower productivity and longer turnover times. Variations in plant productivity, unfavourable conditions for litter decomposition due to poor quality, and nutrient limitations due to low pH, may be of particular importance in determining the small scale spatial variability, long MRT, and preferential accumulation of POM. Failing to incorporate this interplay of controlling factors into models may impair the performance of models to project SOM responses to environmental change.

13.7.3 Plant community and litter quality

Litter quality is important in determining SOM formation (Scholes *et al*., 1997), and different plant tissues and their chemical composition influence SOM decomposition (Oades, 1988). Indeed, litter quality has been shown as a strong indicator of decomposition in northern European mountain soils (Cornelissen *et al*., 2007). The observed site to site variability in decomposition of the Swiss alpine sites in this study may thus be related to differences in plant communities. The most notable difference in vegetation species was observed between the top elevation site, which is dominated by dwarf shrubs and a comparatively large proportion of lichens, and at the middle site which has a larger proportion of legumes compared to the other sites (Figure 25). This suggests that the quality of plant residue entering the soil may differ considerably. Dwarf shrub litter, particularly of *Ericaceae*, is considered to be of low decomposability (Springob and Kirchmann, 2002). The relatively high abundance of dwarf shrubs at the uppermost site, in combination with effects of low pH, may contribute to the long soil C residence time observed. At the middle site, the plant community reflects the higher pH observed, as indicated by the Ellenberg value (Figure 26). In addition to its effects on SOM turnover (see above), soil pH has important implications for plants through nutrient availability and exoenzyme activity (Kalburtji *et al*., 1997; Kok and Vandervelde, 1991; Griffith *et al*., 1995).

The high abundance of legumes at the middle site could provide additional N through the activity of N_2 fixing bacteria. Consistent differences in C/N ratios indicate higher decomposition rates of labile material at the middle site (Table 10). This may be related to the availability of N which may be highest at the middle site and particularly low at the uppermost sites. Much shorter MRTs across all depths at the middle site indicate an accelerated SOM turnover. However, as the SOC content is similar to other sites with longer MRTs, the rapid turnover is compensated by larger litter input from the productive vegetation. This interpretation is corroborated by the highest proportion of labile C in 0-30 cm (Table 5), indicative for a high throughput of plant residues.

13.8 Root densities and turnover trends with elevation

This Pyrenean limestone grassland gradient contains similar root densities (2-15 t ha^{-1}) in 0-20 cm as those in grassland soils on siliceous bedrock in the Alps. Root densities of 15 t ha^{-1} at the highest limestone grassland site (2293 m asl) are similar to those of a similar elevation in the siliceous grassland soils where at the lowest site (2285 m asl) root densities to 20 cm were also calculated as 15 t ha^{-1}. Leifeld *et al*. (2009) reported root densities of 4-11 t ha^{-1} in 0-20 cm along a comparable elevation gradient of 810-2200 m asl on siliceous bedrock, which increased with elevation. Root densities in a separate study carried out at the location of the microbial translocation cores in this study, along a Swiss grassland elevation gradient of 1665-2525 m asl, ranged from 11-22 t ha^{-1} in the top 20 cm (Hitz *et al*., 2001). In the alpine grassland soils of this study, root dry matter densities were higher than those of lower elevation soils, reaching 42 t ha^{-1} at 2564 m asl. Together, these data indicate an increase in grassland root densities with elevation, independent of bedrock. High root densities in alpine soils may be a substantial source of labile C input and could therefore be an important indicator for future C stocks in such soil systems (Hitz *et al.*, 2001). However, how quickly this root C can be incorporated into SOM is dependant on the turnover rate.

While no trend with depth (between 0-30 cm) was indicated in roots from the siliceous alpine site 2564 m asl, long turnover times of c. 14 years were indicated at this elevation. Root turnover times from these limestone grassland soils increased significantly with elevation, reaching 8 years at the uppermost site of 2293 m asl. Root turnover times also increased in siliceous subalpine and alpine grasslands of the Alps from 3 years at 1665 m asl. to c. 8 years at 2525 m asl (Hitz *et al*., 2001). It is notable that these values, which are similar to those reported in this limestone grassland gradient, are similar despite the application of a different method used in the calculation of root turnover times. In combination, these results suggest that root turnover times increase with elevation, and thus decreasing temperature, in grasslands of various geology. Indeed, increasing root turnover times in correspondence with decreasing MAT has been reported along a MAT gradient (-23-28 °C) compiled from an assembled database of almost 200 published documents carried out worldwide (Gill and Jackson, 2000).

13.9 Soil microbial community

13.9.1 Site factors influencing microbial distribution

Biomass and composition of soil microbial communities differ between (sub)alpine sites of the Vereina valley, thus reflecting differences between sites in several biotic and abiotic factors. Because these factors act in concert, it remains difficult to relate differences in microbial communities to individual factors such as temperature. PCA analysis reveals that soil depth, site, pH and substrate composition are important drivers for the community composition. Site, as a surrogate for temperature, is mainly expressed in PC2 where loadings indicate site effects to be particularly strong for those individual PLFAs that correspond to EM fungi and actinomycetes. Along PC2, the translocated cores are placed between Jöri and Vereina. Factors such as pH and substrate composition, however, seem to outweigh the influence of temperature itself and correlate with PC1 and PC3. In the Vereina valley, none of these factors is correlated with elevation.

13.9.2 Influence of soil depth

Among the measured parameters, some tend to be rather resilient and show little difference between sites, including TMB in the upper soil layer and many of the

individual PLFAs, whereas others show distinct differences. Among the latter are fractions of TMB of several structural groups, Gram(+) bacteria, and TMB in the lower soil layer studied here. The data suggest that changes in microbial communities in response to environmental conditions are more pronounced in the lower soil layer however this may be a reflection of the greater variation of basic soil properties among 10-20 cm than among 0-10 cm layers (Figure 29; Table 3).

Differences in microbial communities between soil layers, as observed in three of the four (sub)alpine grassland sites, have also been observed in other environments. For instance, steep declines in total microbial biomass with soil depth has also been reported in Mediterranean, temperate and alpine grassland soils (Fierer *et al*., 2003; Allison *et al*., 2007; Imberger and Chiu, 2001). This was not the case at the Vereina site, where soil microbial biomass was similar at both depths, in line with the soil C concentration which was also similar at both soil depths. This characteristic, in addition to significantly higher C concentrations suggest a strong difference between the local Vereina soil and the other soils studied.

13.9.3 Site characteristics indicate limited decomposition

In comparison to the other sites, the lowest microbial biomass content was found at Vereina. The lack of microbial activity despite high C content is reflected in the high soil C/N ratios (indicating material which is less decomposed) particularly in 10-20 cm. High soil C concentrations and wide C/N ratios for that site were also reported in the previous study (Egli *et al*., 2004) and indicate hindered decomposition of below-ground plant residues. High soil C concentrations and wide C/N ratios were also found in the Furka pass alpine grassland soils and have also been reported in other alpine grassland soils, for example Hitz *et al*. (2001) reported wide C/N ratios of between 30 and 70, this could therefore be a typical characteristic of alpine grassland soils due to their high POM and litter fraction. An effect of substrate composition was also indicated by PC3. This confirmed that the conditions of the local Vereina soil differed considerably from those of the other natural soils (Jöri, Stutzegg), and also from the soil in the translocated cores. Soil moisture strongly influences soil microbial communities (Schimel *et al*., 1999; Wilkinson *et al*., 2002). The Vereina site did indicate the highest proportion of cyclopropane Fatty acids, which have previously been shown to be an indicator for anaerobic bacteria (Vestal and White, 1989; Ratledge and Wilkinson, 1988). Therefore, low biomass content at the Vereina site may be a result of factors limiting microbial activity such as an anoxic environment. Although soil moisture of the Vereina site was previously classified as similar to Jöri (Egli *et al*., 2004), visual inspection during sampling indicated higher soil moisture. This is supported by presence of *C. fusca* at this site which indicates temporary high soil moisture content. The interpretation of these results are that they are indicative for partially anaerobic conditions at this site which, despite higher temperatures, result in higher C concentrations and a different substrate composition than at the higher elevation site.

13.9.4 Effect of soil core translocation

The different environment at the Vereina site resulted in soil microbial biomass contents which were significantly different from the other sites. Translocation of soil cores from a higher elevation site to this site resulted in a shift in the microbial communities, but soil C concentrations in these cores remained similar to their site of origin. Therefore changes observed in the microbial community are likely a result of a change in climate and in the corresponding vegetation. Unlike the Vereina site, the Stutzegg site has similar C concentrations to the Jöri site but is also at a lower elevation and therefore experiences higher air temperatures. Comparison of microbial communities between the Jöri site/ Jöri-Vereina cores/ Stutzegg site show that the microbial biomass content at both soil depths, the proportion of specific biomarkers, the

proportions of all structural groups at lower depths and some structural groups in the upper depths indicate a trend from the high elevation site towards the lower elevation site. This shift may be driven by temperature, whereas the difference between the cores and the local soil at Vereina suggest the influence of additional factors such as soil moisture and vegetation.

13.9.5 Soil microbial group and ratio trends

An increasing AM fungal proportion with elevation in alpine soils has been reported previously (Margesin *et al*., 2009). In contrast, decreasing fungal diversity with elevation has been observed (Schinner and Gstraunthaler, 1981). In this study an increasing proportion of EM fungi was indicated with elevation, while AM fungi decreased with elevation at both soil depths. Without considering the local Vereina soil, these changes with elevation could reflect the influence of different soil temperatures with higher temperatures favouring AM fungi. Also, AM fungal proportions generally decrease downwards through the soil profile, which is a trend that has also been reported in forest soil profiles (Richter and Markewitz, 1995; Ekelund *et al*., 2001) indicating less favourable conditions for AM fungi at lower soil depths. In addition, abiotic site conditions exert control on vegetation communities which in turn affect the abundance of AM and EM fungi. Increasing cyFA/monoenoic precursors ratios have been shown to indicate nutritional stress in bacterial communities and have also been associated with decreasing C availability and decreasing microbial activity (Bossio and Scow, 1998; Guckert *et al*., 1986). Indeed, at these sites, this ratio increased with soil depth in the translocated cores and at the Stutzegg and Jöri site, in accordance with decreasing soil C concentrations and decreasing microbial biomass. Additionally, this ratio decreased with soil depth at the Vereina site, in accordance with an increasing soil C concentration and a similar microbial biomass. The availability of C substrates is an important factor in the activity of soil microbial community, however, the role of other factors such as soil moisture, oxygen availability and nutrient availability may also play more important roles in these soils.

13.9.6 Relevance of microbial community findings with global warming

Subalpine and alpine environments are characterised by extreme climatic conditions and are likely to be especially sensitive to changing environmental conditions such as global warming. The microbial community in soils across a (sub)alpine grassland elevation gradient could be a good indicator of present-day climate influence and future induced changes. The principal findings are the following:

- Simulated climate warming through soil translocation induced a shift of the total microbial biomass towards that of lower elevations, with a slight reduction at 0-10 cm soil depth and a significant increase at 10-20 cm soil depth.
- PLFA analyses confirmed that significant differences in microbial communities exist between the sites. The proportions of all structural groups at lower soil depth and some structural groups in the upper depth indicate a trend from the high elevation site towards the lower elevation site which may be driven by combined temperature-vegetation effects.
- Across these three (sub)alpine grassland sites, EM fungi proportions increased with elevation while AM fungi decreased.
- High soil C concentrations and low microbial content at one of the sites indicated factors other than air temperature and C availability were more influential for microbial activity and decomposition.

14 Conclusions

14.1 Respective to aims

Determine C storage and distribution in alpine grassland soils

From siliceous soil samples sampled across a small elevation gradient of an alpine grassland, it was determined that:

(1) Labile C proportions of alpine grassland sites were larger than those reported in temperate grasslands and therefore such alpine grassland soils may be potential C hotspots due to this large proportion of readily degradable C.

(2) While alpine grassland labile C proportions indicated an increasing trend with elevation when compiled with temperate grasslands, this small elevation gradient actually indicated a general decrease of labile C proportion with elevation above 2381 m asl.

(3) While C distribution in these alpine soils indicate large proportions of labile C, C MRTs of bulk soil, root/litter and POM fractions were long and therefore indicate slow turnover in these soils. Increased productivity at single site of this small gradient corresponded to shorter MRT's and therefore similar C contents as the less productive sites.

Does C storage increase with elevation in limestone grassland soils?

From the measurement of total and labile SOC contents across a Pyrenean limestone grassland elevation gradient, it was determined that:

(1) SOC contents (0-20 cm) increase significantly with elevation across this limestone grassland gradient from 853-2293 m asl.

(2) Labile C proportions did not increase with elevation, or show any trend with elevation from temperate to alpine limestone grasslands

Explore which factors are involved in C storage and distribution of alpine soils

No trend with elevation was indicated with C storage across this small Swiss siliceous grassland alpine gradient, however, SOC contents significantly correlated with stone and root densities indicating that physical storage capacity at individual sites may play a more important role than variation in elevation (and therefore temperature) between sites in these alpine soils.

While C distribution in these alpine soils indicated large proportions of labile C in comparison to temperate sites, proportions did not increase significantly with elevation across this small alpine gradient and indicated an maximum proportion of 57.6 % at 2379 m asl before actually indicating a general decrease with increasing elevation. In contrast, mOM C proportions increased with elevation above 2379 m asl, as did mOM C MRTs. While no correlation was found between either POM C or mOM C proportion with any other soil or site factor measured, this pattern may be a result of the longer mOM MRTs indicated at higher elevations, which results in an increase in accumulation of mOM.

What is the long term response of alpine grassland microbial communities to change in environmental conditions?

From a translocation study in the Swiss Alps, it was determined that:

(1) The long-term effect of translocation of soil cores from a higher to a lower elevation site indicates a moderate response of the microbial community towards the new environment 11 years after translocation.

(2) No trends were indicated in the Gram(+) bacteria community with elevation across a gradient of (sub)alpine grassland soils in the Swiss Alps, however fungal communities, relative to TMB, did indicate a trend with EM fungi proportions decreasing with elevation at lower depths while AM fungi increased with elevation in both soil depths (0-10 and 10-20 cm). Furthermore, TMB at 10-20 cm soil depth and the fractional contents of several FA groups, such as pUS, also indicated trends with elevation.

14.2 Relative to hypotheses

Labile C Proportions

As previous studies in temperate siliceous grassland soils indicated increasing labile C proportion with elevation (Leifeld *et al*., 2009; Zimmermann *et al*., 2007), the hypothesis was:

(1) Compared to lower elevation soils, alpine siliceous grassland soils would indicate large proportions of labile C, which increased with elevation

In accordance with the hypothesis, high proportions of labile C were confirmed in these siliceous grassland alpine soils compared with temperate soils. However, while a significant increase in labile C proportion with elevation was indicated, when these alpine soil data were compiled with temperate soil data, this small elevation alpine gradient actually indicated a decrease in labile C proportion after 2379 m asl.

(2) Labile C proportion would increase with elevation from temperate to alpine sites across a limestone grassland elevation and comprise more than 30% of total SOC at elevations above 2000 m asl

In contrast to the hypothesis, labile C proportions across the Pyrenean limestone gradient did not indicate any trend with elevation and only comprised 13.7 % of total SOC at the alpine grassland site (2293 m asl).

Total C contents

From data published in previous studies of limestone grasslands (Garcia-Pausas *et al*., 2007) and siliceous grasslands (references), the hypothesis was that SOC contents would not indicate any trend with elevation across the elevation gradients in either soil type.

In contrast to the hypothesis based on previous work, data from the Pyrenean limestone grassland gradient in this study indicated that SOC contents increased significantly with elevation.

Soil Microbial communities

As a previous study indicated an alteration in below-ground phytomass after 3 years translocation (Egli *et al*., 2004), the hypothesis was that after longer than a decade of translocation:

(1) Soil microbial communities in translocated cores would be different from that at the original site but would not yet have reached the structure of the new site

After over 10 years in a new environment, soil microbial communities in the cores translocated from a high elevation site to a lower elevation site show a moderate shift in structure and activity towards communities at lower and thus warmer elevations.

(2) Soil microbial communities would indicate trends with elevation

With respect to the initial hypothesis, the results of this translocation study confirm that significant differences in microbial communities exist between sites. Consistent trends along the elevation gradient, such as TMB at 10-20 cm soil depth, the fraction of EM

and AM fungi relative to TMB, suggest that some of these differences can be related to variation in temperature.

14.3 Alpine soils as hotspots with climate warming

With respect to climate warming and projected temperature rises, this study indicates that while MAT may play a role in labile C accumulation of siliceous soils, it is not certain whether there is a direct consequence of the low temperatures typical for alpine environments because a similar trend was not observed on the limestone gradient. Data from this study suggests that other factors, such as soil pH, geology or soil characteristics determined by the plant community, as well as the litter quality as related to the plant community, may play more influential roles. Furthermore, no MAT effect was indicated in labile C proportions of limestone soils and therefore soils of this parent materials may be less susceptible to changes in MAT and more strongly driven by other variables. In the case of this limestone Pyrenean grassland elevation gradient, this apparent independence of labile C from MAT may be due to physical soil properties, such as the higher clay content, or it may be due to chemical soil properties such as the higher pH observed.

The slow response of microbial communities observed in the translocated cores could be an important observation when considering soil C activity in a changing climate; (sub)alpine soils may play a particularly important role in response to global warming as temperature effects are predicted to be greater than in temperate regions (Meehl *et al*., 2007; Rebetez and Reinhard, 2008). However, as the site conditions and soil microbial community in the new area of translocation were so different from the other sites, soil C concentrations in the translocated cores remained similar to their site of origin and were still different to those of the new location.

Overall, while large quantities of labile C have been indicated in some alpine soils, due to long turnover times, slow microbial responses and the important influence of factors other than climate, it is unlikely that these alpine grassland soils are climate warming hotspots or will result in strong positive feedbacks to warming.

14.4 Key findings

1 Siliceous alpine grassland soils indicate large proportions of labile C in comparison to temperate soils whereas limestone soils do not

2 While labile C proportion does indicate a significant increase with elevation from temperate to alpine siliceous grassland soils, labile C proportions indicated a decrease with elevation after 2379 m asl

3 C stocks in limestone grassland soils indicate a significant increase with elevation while C stocks in siliceous soils show no trend

4 Strongly acidic siliceous alpine grassland soils indicate long C turnover times and slow productivity

5 Soil microbial community in alpine soils indicate slow response to simulated climate warming

6 Elevation gradients indicate that climate may play a significant role in C distribution of grassland soils however other factors, such as soil type and pH, may play more important roles

7 Data from this study indicates that it is unlikely that alpine soils are C hotspots in the event of climate warming

15 References

Allison, V.J., Yermakova , Z., Millera, R.M., Jastrowa, J.D., Matamalaa, R. 2007. Using landscape and depth gradients to decouple the impact of correlated environmental variables on soil microbial community composition. Soil Biology and Biochemistry 39, 505-516.

Alvarez, R. and Lavado, R.S. 1998. Climate, organic matter and clay content relationships in the Pampa and Chaco soils, Argentina. Geoderma 83, 127– 141.

Amann, R., Ludwig, W., Schleifer, K.H. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiology Review 59, 143-149.

Ammann, C., Spirig, C., Leifeld, J., Neftel, A. 2009. Assessment of the nitrogen and carbon budget of two managed temperate grassland fields. Agriculture, Ecosystems and Environment 133, 150-162.

Arrouays, D., Vion, I., Kicin, J.L. 1995. Spatial analysis and modelling of topsoil carbon storage in temperate forest humic loamy soils of France. Soil Science 159, 191–198.

Baisden, W.T., Amundson, R., Cook, A.C., Brenner, D.L. 2002. Turnover and storage of C and N in five density fractions from California annual grassland surface soil. Global Biogeochemical Cycles 16, 1117–1132.

Baldock, J. A., Oades, J. M., Nelson, P. N., Skene, T. M., Golchin, A., Clarke, P. 2007. Assessing the extent of decomposition of natural organic materials using solid-state 13C NMR spectroscopy. Australian Journal of Soil Research 35, 1061–1083.

Balesdent, J., Wagner, G.H., Mariotti, A. 1988. Soil organic matter turnover in long-term field experiments as revealed by carbon-13 natural abundance. Soil Science Society of America Journal 52, 118-124.

Balesdent, J., Besnard, E., Arrouays, D., Chenu, C. 1998. The dynamics of carbon in particle-size fractions of soil in a forest-cultivation sequence. Plant Soil 201, 49-57.

Balser, T.C. and M.K. Firestone. 2005. Linking microbial community composition and soil processes in a California annual grassland and a mixed-conifer forest. Biogeochemistry 73, 395-415.

Balser, T.C. and Wixon, D.L. 2009. Investigating biological control over soil carbon temperature sensitivity. Global Change Biology 15, 2935–2949.

Baritz, R., De Neve, S., Barancikova, G., Gronlund, A., Leifeld, J., Katzensteiner, K. *et al.,*2004. Land use practices and soil organic matter. In: Reports of the Technical Working Groups Established Under the Thematic Strategy for Soil Protection (eds L. Van-Camp, B. Bujar-Rabal, A.-R. Gentile, R.J.A. Jones, L. Montanarella, C. Olazabal and S.-K. Selvaradjou), pp. 439–465. EUR 21319 EN/3, 872 pp. Office for Official Publications of the European Communities, Luxembourg.

Batjes, N.H. and Sombroek, W.G. 1997. Possibilities for carbon sequestration in tropical and subtropical soils. Global Change Biology 3, 161–173.

Bavay, M., Lehning, M., Jonas, T., Lowe, H. 2009. Simulations of future snow cover and discharge in Alpine headwater catchments. Hydrological Processes 23, 95–108.

Beniston, M. 2006. Mountain weather and climate: A general overview and a focus on climatic change in the Alps. Hydrobiologia 562, 3-16.

Beniston, M., Keller, F., Koffi, B., Goyette, S. 2003. Estimates of snow accumulation and volume in the Swiss Alps under changing climatic conditions. Theoretical and Applied Climatology 76, 125–140.

Berg, B. and Meentemeyer, V. 2002. Litter quality in a north European transect versus carbon storage. Plant and Soil 242, 83–92.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911-917.

Bossio, D.A. and Scow, K.M. 1998. Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecology 35, 265–278.
Bradford, M.A., Davies, C.A., Frey, S.D., Maddox, T.R., Melillo, J.M., Mohan, J.E., Reynolds, J.F., Treseder, K.K., Wallenstein, M.D. 2008. Thermal adaptation of soil microbial respiration to elevated temperature. Ecology Letters 11, 1316-1327.
Budge, K., Leifeld, J., Hiltbrunner, E., Fuhrer, J. 2010. Litter quality and soil pH are strong drivers of carbon turnover and distribution in alpine grassland soils. Biogeosciences Discussion 7, 6207-6242.
Bunnell, F. L., Tait, D. E. N., Flanagan, P. W., Cleve, K. V. 1977. Microbial respiration and substrate weight loss: I. A general model of the influence of abiotic variables. Soil Biology and Biochemistry 9, 33–40.
Buyanovsky, G. A., Aslam, M., Wagner, G. H. 1994. Carbon turnover in soil physical fractions. Soil Science Society of America Journal 58, 1167-1173.
Cambarella, C.A. and Elliott, E.T. 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Science Society of America Journal 56, 777-783.
Cambardella, C. A. and Elliott, E. T. 1994. Carbon and nitrogen distribution in aggregates from cultivated and native grassland soils. Soil Science Society of America Journal 57, 1071-1076.
Chan, K. Y. 2001. Soil particulate organic carbon under different land use and management. Soil Use and Management 17, 217–22.
Cornelissen, J.H.C., van Bodegom, P.M., Aerts, R. *et al*. 2007. Global negative vegetation feedback to climate warming responses of leaf litter decomposition rates in cold biomes. Ecological Letters 10, 619-627.
DeGrood, S.H., Claassen, V.P., Scow, K.M. 2005. Microbial community composition on native and drastically disturbed serpentine soils. Soil Biology and Biochemistry 37, 1427–1435.
Djukic, I., Zehetner, F., Mentler, A., Gerzabeck, M.H. 2010a. Microbial community composition and activity in different Alpine vegetation zones. Soil Biology and Biochemistry 42, 155-161.
Djukic, I., Zehetner, F., Tatzber, M., Gerzabek, M.H. 2010b. Soil organic-matter stocks and characteristics along an Alpine elevation gradient. Journal of Plant Nutrition and Soil Science 173, 30–38
Dullinger, S., Dirnbö ck, T., Grabherr, G. 2003. Patterns of shrub invasion into high mountain grasslands of the Northern Calcareous Alps, Austria. Arctic, Antarctic, and Alpine Research 35, 434-441.
EDI (Eidgenössisches Departement des Innern), 1992. Hydrologischer Atlas der Schweiz. Landeshydrologie und -geologie, Bern, Switzerland.
Egli, M., Hitz, C., Fitze, P., Mirabella, A. 2004. Experimental determination of climate-change effects on above-ground and below-ground organic matter in alpine grasslands by translocation of soil cores. Journal of Plant Nutrition and Soil Science 167, 457-470.
Ekelund, F., Ronn, R., Christensen, S. 2001. Distribution with depth of protozoa, bacteria and fungi in soil profiles from three Danish forest sites. Soil Biology and Biochemistry 33, 475–48.
Eliasson, P.E., Mcmurtrie, R.E., Pepper, D.A., Strömgren, M., Linder, S., Ågren, G.I., 2005. The response of heterotrophic CO2 flux to soil warming. Global Change Biology 11, 167-181.
Ellenberg, H. 1988. Vegetation Ecology of Central Europe, Cambridge University Press, Cambridge.
Ertsten, A. C. D., Alkemade, J. R. M., Wassen, M. J. 1998. Calibrating Ellenberg indicator values for moisture, acidity, nutrient availability and salinity in the Netherlands. Plane Ecology 135, 113-124.

Evgrafova, S.Y., Santruckova, H., Shibistova, O.B., Elhottova, D., Cerna, B., Zrazhevskaya, G.K., Lloyd, D. 2008. Phospholipid fatty acid composition of microorganisms in pine forest soils of central Siberia. Biology Bulletin 35, 452-258.

FAL, 1998. Methodenbuch für Boden-, Pflanzen- und Lysimeterwasser-untersuchungen. Schriftenreihe der FAL 27, Eidgenössische Forschungsanstalt für Agrarökologie und Landbau, Zürich-Reckenholz, Schweiz.

Feller, C. and Beare, M.H. 1997. Physical control of soil organic matter dynamics in the tropics. Geoderma 79, 69–119.

Fierer, N., Schimel, J.P., Holden, P.A. 2003. Variation in microbial community composition through two soil depth profiles. Soil Biology and Biochemistry 35, 167-176.

Finzi, A.C., Breemen, N.V., Canham, C.D. 1998. Canopy tree soil interactions within temperate forests: tree species effects on carbon and nitrogen. Ecological Applications 8, 440– 446.

Foereid, B., Barthram, G.T., Marriott, C.A. 2007. The CENTURY model failed to simulate soil organic matter development in an acidic grassland. Nutrient Cycling in Agroecosystems 78, 143–153.

Frey, S.D., Drijber, R., Smith, H., Melillo, J. 2008. Microbial biomass, functional capacity, and community structure after 12 years of soil warming. Soil Biology and Biochemistry 40, 2904-2907.

Friedlingstein, P., Cox, P., Betts, R., Bopp, L., von Bloh, W., Brovkin, V., Cadule, P., Doney, S., Eby, M., Fung, I., Govindasamy, B., John, J., Jones, C., Joos, F., Kato, T., Kawamiya, M., Knorr, W., Lindsay, K., Matthews, H. D., Raddatz, T., Rayner, P., Reick, C., Roeckner, E., Schnitzler, K. G,, Schnur, R., Strassmann, K., Weaver, A. J., Yoshikawa, C., Zeng, N. 2006. Climate–carbon cycle feedback analysis: results from the C4MIP model intercomparison. Journal of Climate 19, 3337-3353.

Frostegård, Å., Tunlid, A., Bååth, E. 1993a. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Applied and Environmental Microbiology 59, 3605-3617.

Frostegård, Å., Bååth, E., Tunlid, A. 1993b. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology and Biochemistry 25, 723-730..

Frostegård, Å., Tunlid, A., Bååth, E. 1996. Changes in microbial community structure during long-term incubation in two soils experimentally contaminated with metals. Soil Biology and Biochemistry 28, 55-63.

Gabathuler, M. 1999. Physical ecosystem determinants in high mountain lakes. The Jöri-lakes, Switzerland. Ph.D. thesis, ETH No. 13449, ETH Zürich, Switzerland.

Ganuza, A. and Almendros, G. 2003. Organic carbon storage of the Basque Country (Spain): the effect of climate, vegetation type and edaphic variables. Biology and Fertility of Soils 37, 154-162.

Garcia-Pausas, J., Casals, P., Camarero, L., Huguet, C., Sebastià, M.T., Thompson, R., Romanyà, J. 2007. Soil organic carbon storage in mountain grasslands of the Pyrenees: effects of climate and topography. Biogeochemistry 82, 279-289.

Gee, G.W. and Bauder, J.W. 1986. Particle-size Analysis, in: Page, A.L. (Ed.). Methods of soil analysis, Part1, Physical and mineralogical methods, Second Edition, Agronomy Monograph 9, American Society of Agronomy, Madison, WI, 383-411.

Gill, R.A. and Jackson, B.B. 2000. Global patterns for root turnover in terrestrial ecosystems. New Phytologist 147, 13-31.

Golchin, A., Oades, J.M., Skjemstad, J.O., Clarke, P. 1994a. Study of free and occluded particulate organic matter in soils by solid state 13C CP/MAS NMR spectroscopy and scanning electron microscopy. Australian Journal of Soil Research 32, 285–309

Golchin, A., Oades, J.M., Kjemstad, J.O., Clarke, P. 1994b. Soil structure and carbon cycling. Australian Journal of Soil Research 32, 1043–1068.

Grandy, A. S., Strickland, M. S., Lauber, C., Bradford, M. A., Fierer, N. 2009. The influence of microbial communities, management, and soil texture on soil organic chemistry. Geoderma 150, 278–286.
Griffith, M.B., Perry, S.A., Perry, W.B. 1995. Leaf liner processing and exoenzyme production on leaves in streams of different pH, Oecologia 102, 460–466.
Guckert, J.B., Antworth, C.P., Nichols, P.D., White, D.C. 1985. Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. FEMS Microbiological Ecology 31, 147–158.
Guckert, J.B., Hood, M.A., White, D.C. 1986. Phospholipid, ester-linked fatty acid profile changes during nutrient deprivation of Vibrio cholerae: increase in trans/cis ratio and proportion of cyclopropyl fatty acids. Applied and Environmental Microbiology 52, 749–801.
Haack, S.K., Garchow, H., Odelson, D.A., Forney, L.J., Klug, M.L., 1994. Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities. Applied and Environmental Microbiology 60, 2483–2493.
Hagedorn, F., Mulder, J., Jandl, R. 2010. Mountain soils under a changing climate and land-use. Biogeochemistry 97, 1-5.
Hakkenberg, R., Churkina, G., Rodeghiero, M., Börner, A., Steinhof, A., Cescatti, A. 2008. Temperature sensitivity of the turnover times of soil organic matter in forests. Ecological Applications 18, 119–131.
Harkness, D.D., Harrison, A.F., Bacon, P.J. 1986. The temporal distribution of 'Bomb' 14C in a forest soil. Radiocarbon 28, 328-337.
Harte, J., Saleska, S., Shih, T. 2006. Shifts in plant dominance control carbon-cycle responses to experimental warming and widespread drought. Environmental Research Letters, 1 Art. No. 014001.
Hattenschwiler, S., Tiunov, A.V., Scheu, S. 2005. Biodiversity and litter decomposition in terrestrial ecosystems. Annual Review of Ecology, Evolution, and Systematics 36, 191–218.
Hawkes, J.C., Pyatt, D.G., White, I.M.S. 1997. Using Ellenberg values to assess soil quality in British forests from ground vegetation: a pilot study, Journal of Applied Ecology 34, 375-387.
Helfrich, M., Ludwig, B., Buurman, P., Flessa, H. 2006. Effect of land use on the composition of soil organic matter in density and aggregate fractions as revealed by solid-state 13C NMR spectroscopy. Geoderma 136. 331-341.
Hitz, C., Egli, M., Fitze, P., 2001. Below-ground and above-ground production of vegetational organic matter along a climosequence in alpine grasslands. Journal of Plant Nutrition and Soil Science 164, 389-397.
Hobbie, S.E., Schimel, J.P., Trumbore, S.E., Randerson, J.R. 2000. Controls over carbon storage and turnover in high-latitude soils. Global Change Biology 6, 196-210.
Hogberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Hogberg, M.N. *et al*. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411, 789–792.
Hsieh, Y.P. 1996. Soil organic carbon pools of two tropical soils inferred by carbon signatures. Soil Science Society of America Journal 60, 1117–1121.
Imberger, K.T. and Chiu, C.Y. 2001. Spatial changes of soil fungal and bacterial biomass from a sub-alpine coniferous forest to grassland in a humid, sub-tropical region. Biology and Fertility of Soils 33, 105-110.
IPCC, 2007. Climate Change 2007. Impacts, Adaptation and Vulnerability In: Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J. and Hanson, C.E., (Eds.), Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.
Jobbagy, E.G. and Jackson, R.B. 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecological Applications 10, 423-436.

John, B., Yamashita, T., Ludwig, B., Flessa, H. 2005. Organic carbon storage in aggregate and density fractions of silty soils under different types of land use. Geoderma 27, 319-361.
Jones, R.J.A., Hiederer, R., Rusco, E., Montanarella, L. 2005. Estimating organic carbon in the soils of Europe for policy support. European Journal of Soil Science 56, 655-671.
Kalburtji, K.L., Mamolos, A.P., Kostopoulou, S. 1997. Nutrient release from decomposing Lotus corniculatus residues in relation to soil pH and nitrogen levels. Agriculture, Ecosystems and Environment 65, 107-112.
Kemmitt, S.J., Wright, D., Goulding, K.W.T., Jones, D.L. 2006. pH regulation of carbon and nitrogen dynamics in two agricultural soils. Soil Biology and Biochemistry 38, 898-911.
Kelly, J.J., Haggblom, M.M., Tate, R.L., 2003. Effects of heavy metal contamination and remediation on soil microbial communities in the vicinity of a zinc smelter as indicated by analysis of microbial community phospholipid fatty acid profiles. Biology and Fertility of Soils 38, 65-71.
Kief, T.L.R., Ringelberg, D.B., White, D.C., 1994. Changes in ester-linked phospholipids fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. Applied and Environmental Microbiology 60, 3292–3299.
Knicker, H. and Lüdemann, H.D. 1995. N-15 and C-13 CPMAS and solution NMR-studies of N-15 enriched plant material during 600 days of microbial degradation. Organic Geochemistry 23, 329-341.
Knivett, V.A. and Cullen, J. 1965. Some factors affecting cyclopropane acid formation in Escherichia coli. Biochemistry Journal 96, 771-776.
Kögel-Knabner, I. 2002. The macromolecular organic composition in plant and microbial residues as input to soil. Soil Biology and Biochemistry 34, 139-162.
Kok, C.J. and Vandervelde, G. 1991. The influence of selected water-quality parameters on the decay-rate and exoenzymatic activity of detritus of Nymphaea alba L floating leaf blades in laboratory experiments. Oecologia 88, 311–316.
Kölbl, A. and Kögel-Knabner, I. 2004. Content and composition of free and occluded particulate organic matter in a differently textured arable Cambisol as revealed by solid-state C-13 NMR spectroscopy. Journal of Plant Nutrition and Soil Science 167, 45-53.
Körner, C. 2003. Alpine plant life, 2nd edition, Springer, Heidelberg.
Leifeld, J., Bassin, S., Fuhrer, J. 2005. Carbon stocks in Swiss agricultural soils predicted by land-use, soil characteristics, and elevation. Agriculture, Ecosystems and Environment 105, 255–266.
Leifeld, J. and Fuhrer, J. 2009. Long-term management effects on soil organic matter in two cold, high-elevation grasslands: clues from fractionation and radiocarbon dating. European Journal of Soil Science 60, 230–239.
Leifeld, J. and Kögel-Knabner, I. 2005. Soil organic matter fractions as early indicators for carbon stock changes under different land-use? Geoderma 124, 143-155.
Leifeld, J., Zimmermann, M., Fuhrer, J. 2008. Simulating decomposition of labile soil organic carbon: Effects of pH. Soil Biology and Biochemistry 40, 2948-2951.
Leifeld, J., Zimmermann, M., Fuhrer, J., Conen, F. 2009. Storage and turnover of carbon in grassland soils along an elevation gradient in the Swiss Alps. Global Change Biology 15, 668-679.
Linn D.M. and Doran J.W. 1984. Effect of water-tilled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Science Society of America Journal 48, 1257-l 272.
Lipson, D.A. and Schmidt, S.K. 2004. Seasonal changes in alpine soil bacterial community in the Colorado Rocky Mountains. Applied and Environmental Microbiology 70, 2867-2879.

Lovell, R.D., Jarvis, S.C., Bardgett, R.D. 1995. Soil microbial biomass and activity in long-term grassland: effects of management changes. Soil Biology and Biochemistry 27, 969-975.
Luo, Y.Q., Wan, S., Hui, D., Wallace, L. 2001. Acclimatization of soil respiration to warming in a tall grass prairie. Nature 413, 622-625.
Margesin, R., Jud, M., Tscherko, D., Schinner, F. 2009. Microbial communities and activities in alpine and subalpine soils. FEMS Microbiology Ecology 67, 208-218.
McGill, W.B. 1996. Review and classification of ten soil organic matter (SOM) models. pp. 111–132. In: Powlson, D.S., Smith, P. and Smith, J.U. (eds.), Evaluation of Soil Organic Matter Models: Using Existing Long-term Datasets. Springer, New York.
Meehl, G.A., Stocker, T.F., Collins, W.D., Friedlingstein, P., Gaye, A.T., Gregory, J.M., Kitoh, A., Knutti, R., Murphy, J.M., Noda, A., Raper, S.C.B., Watterson, I.G., Weaver, A.J., Zhao, Z.C., Global Climate Projections, in: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, USA.
Meentemeyer, V., Box, E. O., Thompson, R. 1982. World patterns and amounts of terrestrial plant litter production. Bioscience 32, 125-128.
Melillo, J. M., Aber, J. D., Muratore, J. F. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63, 621-626.
Miller, A., Amundson, R., burke, I.C., Yonker, C. 2004. The effect of climate and cultivation on soil organic C and N. Biogeochemistry 67, 57-72.
Neff, J., Townsend, A., Gleixner, G., Lehman, S., Turnbull, J., Bowman, W. 2002. Variable effects of nitrogen additions on the stability and turnover of soil carbon. Nature 419, 915-917.
Ninyerola, M., Pons, X., Roure, J.M. 2005. Atlas Climático Digital de la Península Ibérica. Metodología y aplicaciones en bioclimatología y geobotánica. Universidad Autónoma de Barcelona, Bellaterra, Spain.
Oades, J. M. 1998. The retention of organic matter in soils, Biogeochemistry 5, 35-70.
Olsson, P.A., Bååth, E., Jakobsen, I., Söderström, B. 1995. The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. Mycological Research 99, 623-629.
Olsson, P.A. 1999. Minireview - Signature fatty acids provide tools for determination of distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiology Ecology 29, 303-310.
Osher, L.J., Matson, P.A., Amundson, R. 2003. Effect of land use change on soil carbon in Hawaii. Biogeochemistry 65, 213-232.
Paterson, E., Gebbing, T., Abel, C., Sim, A., Telfer, G., 2007. Rhizodeposition shapes rhizosphere microbial community structure in organic soil. New Phytologist 173, 600-610.
Percival, H.J., Parfitt, R.L., Scott, N.A. 2000. Factors controlling soil carbon levels in New Zealand grasslands: is clay important? Soil Science Society of America Journal 64, 1623-1630.
Pohl, M., Alig, D., Körner, C., Rixen, C. 2009. Higher plant diversity enhances soil stability in disturbed alpine ecosystems. Plant and Soil 324, 91-102.
Ponder, F., Tadros, M., Loewenstein, E. 2009. Microbial properties and litter and soil nutrients after two prescribed fires in developing savannas in an upland Missouri Ozark Forest. Forest Ecology and Management 257, 755-763.
Post, W.M., Emanuel, W.R., Zinke, P.J., Stangenberger, A.G. 1982. Soil carbon pools and world life zones. Nature 298, 156-159.
Ratledge, C. and Wilkinson, S.G. 1988. Microbial lipids. Academic Press, London.
Reth, S., Graf, W., Reichstein, M., Munch, J.C. 2009. Sustained stimulation of soil respiration after 10 years of experimental warming. Environmental Research Letters 4, 1-5.

Rebetez, M., Reinhard, M., 2008. Monthly air temperature trends in Switzerland 1901–2000 and 1975–2004. Theoretical and Applied Climatology 91, 27–34.
Richter, D., Markewitz, D., 1995. How deep is soil? BioScience 45, 600–609.
Rillig, M.C. and Mummey, D.L. 2006. Mycorrhizas and soil structure. New Phytologist 171, 41–53.
Rinnan, R., Michelsen, A., Bååth, E., Jonasson, S. 2007. Fifteen years of climate change manipulations alter soil microbial communities in a subarctic heath ecosystem. Global Change Biology 13, 28-39.
SAEFL 2005. Switzerland's fourth national communication under the UNFCCC. Swiss Agency for Environment, Forests, and Landscape.
Saleska, S.R., Shaw, M.R., Fisher, M.L., Dunne, J.A., Still, C.J., Holman, M.L., Harte, J. 2002. Plant community composition mediates both large transient declines and predicted long-term recovery of soil carbon under global warming. Global Biogeochemical Cycles 16, 1055.
Schimel, J.P., Gulledge, J.M., Clein-Curley, J.S., Lindstrom, J.E., Braddock, J.F., 1999. Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. Soil Biology and Biochemistry 31, 831–838.
Schindlbacher, A., Zechmeister-Boltenstern, S., Jandl, R. 2009. Carbon losses due to soil warming: Do autotrophic and heterotrophic soil respiration respond equally? Global Change Biology 15, 901–913.
Schinner, F. and Gstraunthaler, G., 1981. Adaptation of microbial activities to the environmental conditions in alpine soils. Oecologia 50, 113-116.
Schlesinger, W. and Andrews, J. 2000. Soil respiration and the global carbon cycle. Biogeochemistry 48, 7-20.
Scholes, M.C., Powlson, D., Tian, G. 1997. Input control of organic matter dynamics. Geoderma 79, 25-47.
Schulze, K., Borken, W., Muhr, J., Matzner, E. 2009. Stock, turnover time and accumulation of organic matter in bulk and density fractions of a Podzol soil. European Journal of Soil Science 60, 567-577.
Schwarb, M., Frei, C., Schär, C., Daly, C. 2001. Mean seasonal precipitation throughout the European Alps 1971-1990, Hydrological Atlas of Switzerland, Table 2.7, Swiss Federal Office for the Environment, Berne, Switzerland.
Six, J., Bossuyt, H., Degryze, S., Denef, K. 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil and Tillage Research 79, 7-31.
Sollins, P., Homann, P., Caldwell, B. A. 1996. Stabilization and destabilization of soil organic matter: Mechanisms and controls. Geoderma 74, 65-105.
Spain, A.V. 1990. Influence of environmental conditions and some soil chemical properties on the carbon and nitrogen contents of soil tropical Australian rainforest soils. Australian Journal of Soil Research 28, 825– 839.
Springob, G. and Kirchmann, H. 2002. C-rich sandy Ap horizons of specific historical land-use contain large fractions of refractory organic matter. Soil Biology and Biochemistry 34,1571-1581.
Stump, L.M. and Binkley, D. 1992. Relationships between litter quality and nitrogen availability in Rocky Mountain forests. Canadian Journal of Forest Research 23,1402–1407.
Swiss Confederation, 2005: Switzerland's Fourth National Communication under the UNFCCC. Swiss Agency for the Environment, Forests and Landscape (SAEFL), Berne,
Tian, Y., Ouyang, H., Song, M., Niu, H., Hu, Q. 2008. Distribution characteristics and influencing factors of soil organic carbon in alpine ecosystems on the Tibetan Plateau transect, China. Frontiers of Agriculture in China 2, 404-409.
Tisdall, J. M. and Oades, J. M. 1982. Organic matter and water-stable aggregates in soils. European Journal of Soil Science 33, 141-163.

Trumbore, S.E., Chadwick O.A., Amundson R. 1996. Rapid exchange between soil carbon and atmospheric carbon dioxide driven by temperature change. Science 272, 393–396.

Trumbore S. E. 1997. Potential responses of soil organic carbon to global environmental change. Proceedings of the National Academy of Sciences 94, 8284–8291.

Vestal, J.R. and White, C.D. 1989. Lipid analysis in microbial ecology: Quantitative approaches to the study of microbial communities. Bioscience 39, 535-541.

Vicca, S., Fivez, L., Kockelbergh, F., Van Pelt, D., Segers, J.J.R., Meire, P., Ceulemans, R., Janssens, I. A. 2009. No signs of thermal acclimation of heterotrophic respiration from peat soils exposed to different water levels. Soil Biology and Biochemistry 41, 2014-2016.

Vinton, M.A. and Burke, I.C. 1997. Contingent effects of plant species on soils along a regional moisture gradient in the Great Plains. Oecologia 110, 393-402.

Waldrop, M.P. and Firestone, M.K. 2006. Response of microbial community composition and function to soil climate change. Microbial Ecology 52, 716-724.

Walther, G.R., Beissner, S., Burga, C.A. 2005. Trends in the upward shift of alpine plants. Journal of Vegetation Science 16, 541–548.

Wang, G., Li, Y., Wang, Y., Wu, Q. 2008. Effects of permafrost thawing on vegetation and soil carbon pool losses on the Qinghai-Tibet Plateau, China. Geoderma 143, 143-152.

Wang, L., Ouyang, H., Zhou, C.P., Zhang, F., Song, M.H., Tian, Y.Q. 2005. Soil organic matter dynamics along a vertical vegetation gradient in the Gongga Mountain on the Tibetan Plateau. Journal of Integrative Plant Biology 47, 411-420.

White, D.C., Davis, W.M., Nickels, J.S., King, J.D., Bobbie, R.J. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40, 51-62.

White, D.C., Stair, J.O., Ringelberg, D.B. 1996. Quantitative comparisons of in situ microbial biodiversity by signature biomarker analysis. Journal of Industrial Microbiology 17, 185-196.

Wilke, B.M., Gattinger, A., Frohlich, E., Zelles, L., Gong, P. 2004. Phospholipid fatty acid composition of a 2,4,6-trinitrotolune contaminated soil and an uncontaminated soil as affected by a humification remediation process. Soil Biology and Biochemistry 36, 725–729.

Wilkinson, S.C., Anderson, J.M., Scardelis, S.P., Tisiafouli, M., Taylor, A., Wolters, V., 2002. PLFA profiles of microbial communities in decomposing conifer litters subject to moisture stress. Soil Biology and Biochemistry 34, 189-200.

World Reference Base for Soil Resources (WRB), 2nd Edition. 2006. International Society of Soil Science (ISSS), International Soil Reference and Information Centre (ISRIC) and Food and Agriculture Organization of the United Nations (FAO).

Wu, W., Xiongsheng, Y., Wang, H., Ding, N., Xu, J. 2010. Does history matter? Temperature effects on soil microbial biomass and community structure based on the phospholipid fatty acid (PLFA) analysis. Journal of Soils and Sediments 10, 223-230.

Yamashita, T., Flessa, H., John, B., Helfrich, M., Ludwig, B. 2006. Organic matter in density fractions of water-stable aggregates in silty soils: effect of land use. Soil Biology and Biochemistry 38, 3222-3234.

Yang, Y., Fang, J., Tang, Y., Ji, C., Zheng, C., He, J., Zhu, B. 2008. Storage, patterns and controls of soil organic carbon in the Tibetan grasslands. Global Change Biology 14, 1592-1599.

Zhang, W. and Zhang, H. 2009. Distribution characteristics of soil organic carbon of alpine meadow in the Eastern Qinghai-Tibet Plateau. Wuhan University Journal of Natural Sciences 14, 274-280.

Zimmermann, M., Leifeld, J., Schmidt, M.W.I., Smith, P., Fuhrer, J. 2007. Measured soil organic matter fractions can be related to pools in the RothC model. European Journal of Soil Science 58, 658-667.

Z'graggen, L. and Ohmura, A. 2002. Spatio-temporal variations in net radiation 1984–1993, Hydrological Atlas of Switzerland, Table 4.2, Swiss Federal Office for the Environment, Berne.

Zogg, G., Zak, D., Ringelberg, D., Macdonald, N., Pregitzer, K., White, D. 1997. Compositional and functional shifts in microbial communities due to soil warming. Soil Science Society of America Journal 61, 475-481.

16 Appendixes

16.1 pMC values calculated by AMS measurement of ^{14}C

Fine earth

Location	Site elevation (m asl)	Soil depth (cm)	pMC
Furka pass	2285	0-5	113.75
		5-10	107.22
		10-20	97.11
		20-30	86.23
	2379	0-5	113.89
		5-10	106.02
		10-20	97.54
		20-30	81.18
	2481	0-5	116.47
		5-10	112.27
		10-20	104.64
		20-30	98.08
	2564	0-5	111.50
		5-10	101.00
		10-20	91.08
		20-30	81.07
	2653	0-5	110.67
		5-10	100.49
		10-20	89.29
		20-30	80.47

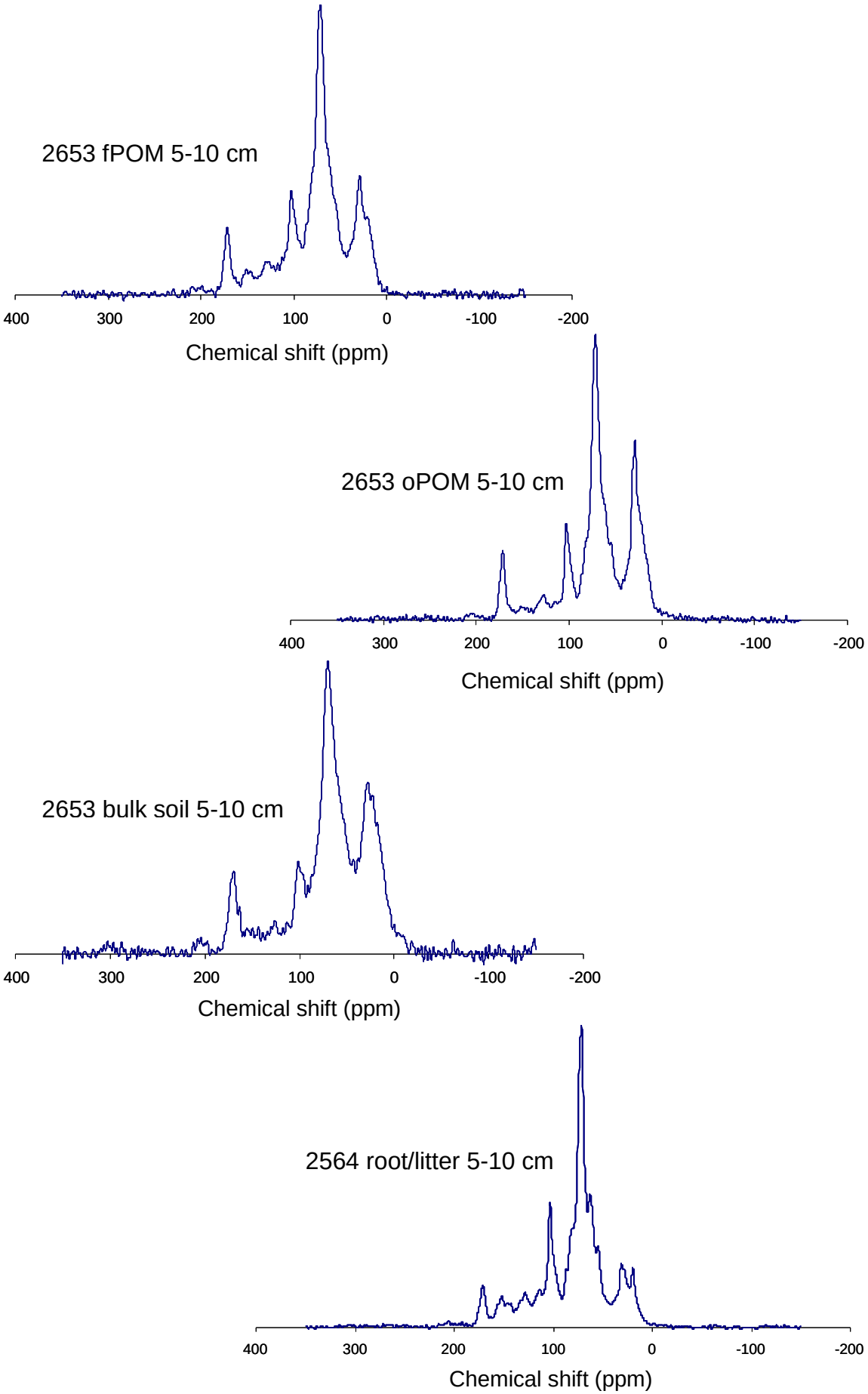
Roots

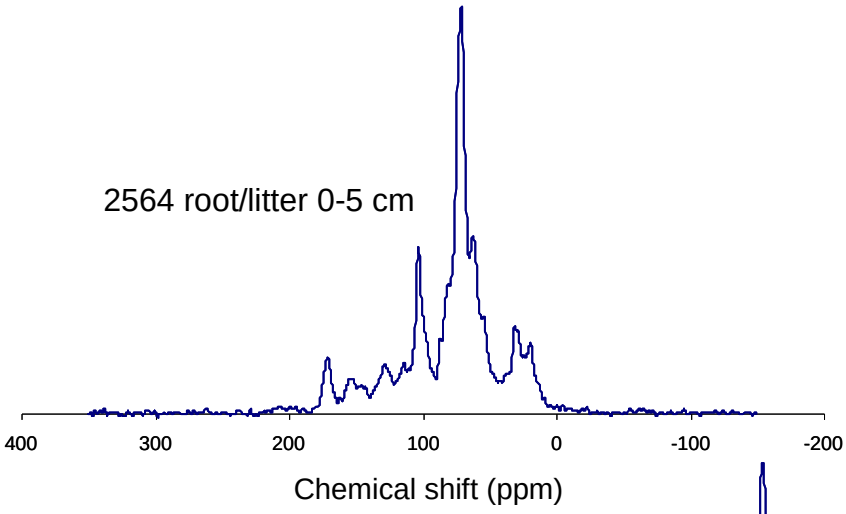
Location	Site elevation (m asl)	Soil depth (cm)	pMC
Furka pass	2564	0-5	114.22
		5-10	113.93
		10-20	114.36
		20-30	112.80
Berguedà	853	0-10	102.16
	1279	0-10	106.38
	1817	0-10	108.72
	2293	0-10	109.33

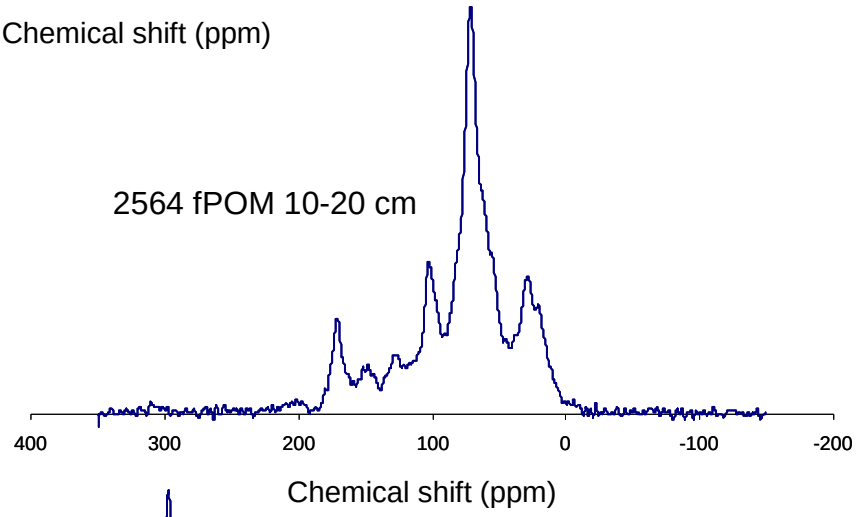
Soil fractions

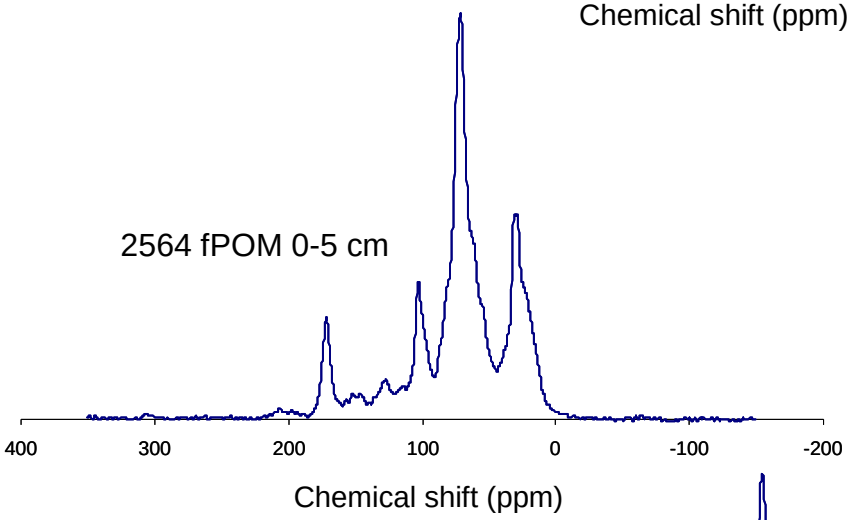
Location	Site elevation (m asl)	Core depth (cm)	Soil fraction	pMC
Furka pass	2285	5-10	Free POM	110.85
		5-10	Occluded POM	109.01
	2379	5-10	Free POM	107.52
		5-10	Occluded POM	106.00
	2564	5-10	Free POM	109.07
		5-10	Occluded POM	106.99
	2653	5-10	Free POM	109.62
		5-10	Occluded POM	107.52
	2564	0-5	Free POM	111.34
		0-5	Occluded POM	108.13
		10-20	Free POM	102.68
		10-20	Occluded POM	95.83
		20-30	Free POM	102.36
		20-30	Occluded POM	90.72

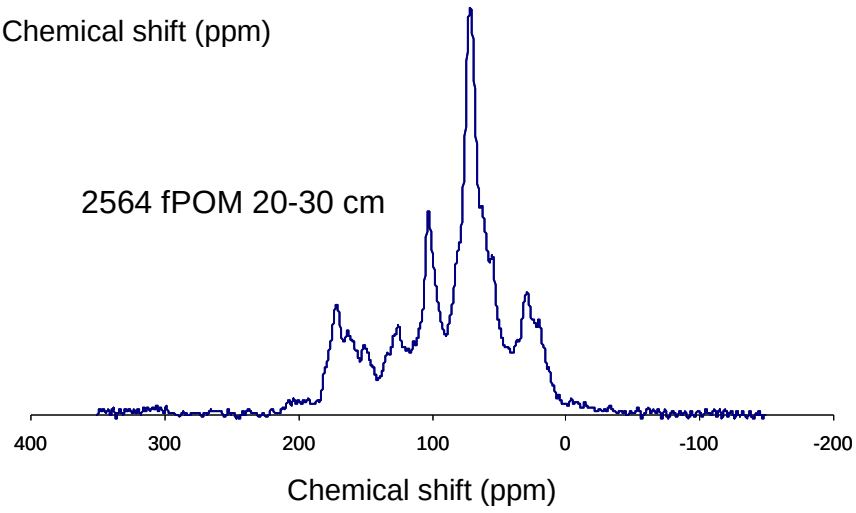
16.2 ^{13}C NMR Spectrum of soil fractions




2564 root/litter 0-5 cm
400
300
200
100
0
-100
-200
Chemical shift (ppm)


2564 fPOM 10-20 cm
400
300
200
100
0
-100
-200
Chemical shift (ppm)


2564 fPOM 0-5 cm
400
300
200
100
0
-100
-200
Chemical shift (ppm)


2564 fPOM 20-30 cm
400
300
200
100
0
-100
-200
Chemical shift (ppm)

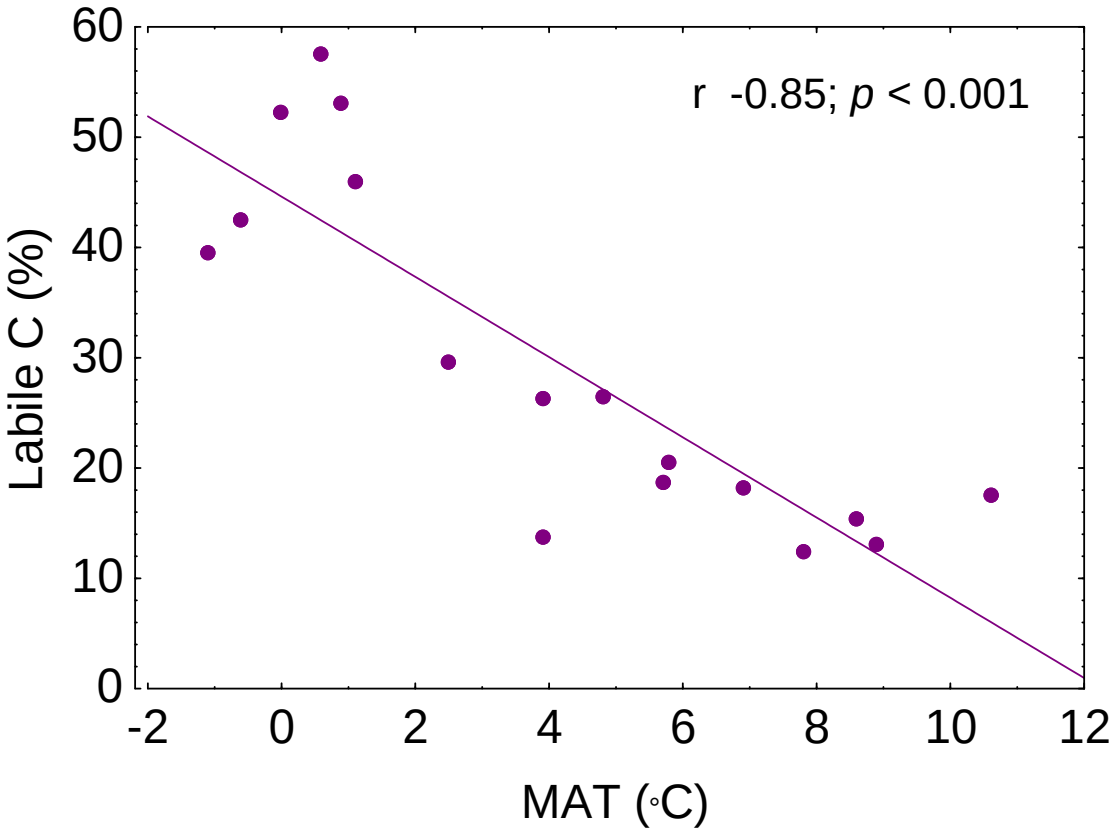
16.3 Furka pass alpine grassland gradient plant species

Achillea pentaphylla
Agrostis schraderiana
Alchemilla millfolium
Anthoxanthum odoratum
Anthyllis vulneraria L.
Caldonia rangiferina
Campanula barbata L.
Campanula scheuchzeri
Carex microglochin
Carex sempervirens
Chamorchis alpina
Cirsium spinosissimum (L.)
Festuca alpina Suter
Festuca violacea aggr.
Galium pumilum
Gentiana punctata L.
Gentiana purpurea
Geum montanum L.
Gnaphalium norvegicum
Gnaphalium supinum L.
Helictotrichon versicolor
Homogyne alpina (L.)
Juncus jacquinii L.
Juncus trifidus L.
Leontodon helveticus
Leontodon hispidus
Ligusticum mutellina
Ligusticum mutellinoides
Loiseleuria procumbens
Lotus corniculatus L.
Lotus corniculatus alpinum
Luzula alpino-pilosa
Myosotis alpestris
Nardus stricta L.
Pedicularis tuberosa L.
Pedicularis verticillata L.
Phleum alpinum L.
Poa alpina L.
Poa chaixii Vill.
Polygonum viviparum L.
Polytrichum sexangulare
Potentilla aurea L.
Pulsatilla vernalis
Ranunculus montanus aggr.
Salix herbacea
Salix retusa L.
Sempervivum montanum L.
Soldanella pusilla
Trifolium alpinum L.
Trifolium badium
Trifolium pallescens
Trifolium pratense L. subsp. Nivale
Vaccinium myrtillus L.
Vaccinium uliginosum
Veronica alpina
Veronica bellidioides

16.4 Mean and SE for PLFA contents (µg/g) of soil from cores and investigation sites at two depths

	Jöri				Jöri-Vereina cores				Vereina				Stutzegg			
PLFA	0-10 cm		10-20 cm		0-10 cm		10-20 cm		0-10 cm		10-20 cm		0-10 cm		10-20 cm	
	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*
i14:0	0.2	*0.0*	0.0	*0.0*	0.2	*0.0*	0.2	*0.0*	0.1	*0.0*	0.1	*0.0*	0.1	*0.0*	0.1	*0.0*
14:0	1.8	*0.2*	0.1	*0.0*	1.5	*0.1*	0.4	*0.1*	0.9	*0.1*	0.7	*0.1*	1.5	*0.2*	0.5	*0.1*
i15:0	15.4	*0.9*	1.3	*0.3*	15.7	*1.5*	3.6	*0.8*	8.8	*0.3*	9.0	*1.1*	17.0	*1.6*	6.9	*1.2*
a15:0	5.8	*0.4*	0.9	*0.3*	6.3	*0.9*	2.2	*0.3*	4.1	*0.3*	4.7	*0.5*	7.0	*0.6*	3.8	*0.4*
15:0	1.4	*0.1*	0.1	*0.1*	1.5	*0.2*	0.2	*0.1*	0.7	*0.1*	0.6	*0.1*	1.0	*0.1*	0.5	*0.0*
i16:1	3.3	*0.3*	0.2	*0.1*	3.0	*0.4*	0.5	*0.1*	0.8	*0.1*	0.4	*0.2*	1.7	*0.2*	1.1	*0.2*
16:1ω11c	0.1	*0.0*	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*
i16:0	11.4	*0.9*	1.7	*0.3*	11.2	*1.1*	2.8	*0.4*	4.2	*0.9*	5.1	*0.6*	7.4	*0.7*	3.5	*0.7*
16:1ω11t	2.2	*0.1*	0.1	*0.1*	1.9	*0.1*	0.4	*0.1*	0.8	*0.0*	0.6	*0.3*	2.3	*0.2*	0.8	*0.1*
16:1ω7c	15.9	*0.5*	1.0	*0.3*	13.8	*1.3*	2.5	*0.6*	4.7	*0.2*	8.3	*1.3*	15.3	*1.8*	5.2	*1.2*
16:1ω5c	7.1	*0.4*	0.5	*0.1*	5.1	*0.5*	0.9	*0.2*	2.7	*0.3*	1.3	*0.2*	6.5	*0.5*	2.0	*0.5*
16:0	40.7	*0.3*	5.2	*1.1*	30.8	*2.9*	8.2	*1.1*	14.6	*1.8*	10.8	*1.5*	35.1	*3.4*	13.8	*2.4*
10-Me-16:0	0.4	*0.1*	0.2	*0.0*	0.2	*0.1*	0.2	*0.0*	0.0	*0.0*	0.3	*0.0*	0.0	*0.0*	0.0	*0.0*
i17:0	3.7	*0.2*	0.3	*0.3*	3.3	*0.4*	0.8	*0.3*	2.8	*0.3*	2.6	*0.1*	2.8	*0.2*	1.8	*0.1*
a17:0	3.7	*0.2*	0.6	*0.2*	3.5	*0.3*	1.1	*0.2*	1.1	*0.5*	1.2	*0.2*	4.5	*0.5*	1.8	*0.3*
12-Me-16:0	2.6	*0.2*	0.3	*0.1*	2.2	*0.1*	0.6	*0.1*	1.1	*0.0*	1.2	*0.2*	2.6	*0.2*	0.8	*0.1*
17:1ω8c	1.6	*0.1*	0.0	*0.0*	1.4	*0.3*	0.1	*0.1*	0.3	*0.0*	0.2	*0.1*	0.9	*0.3*	0.1	*0.0*
17:0cy	4.4	*0.3*	0.8	*0.1*	3.4	*0.2*	1.4	*0.2*	1.8	*0.2*	2.2	*0.2*	5.8	*0.7*	2.1	*0.4*
17:1ω7	0.7	*0.1*	0.0	*0.0*	0.7	*0.0*	0.1	*0.1*	0.3	*0.0*	0.1	*0.1*	1.0	*0.3*	0.1	*0.1*
17:0	0.9	*0.0*	0.0	*0.0*	0.8	*0.4*	0.6	*0.6*	0.1	*0.1*	0.9	*0.5*	0.6	*0.3*	0.2	*0.1*
12-Me-17:0	4.7	*0.3*	0.9	*0.2*	5.3	*0.3*	0.8	*0.4*	1.4	*0.0*	0.2	*0.2*	4.1	*0.3*	2.1	*0.4*
10-Me-17:0	5.4	*0.4*	0.3	*0.1*	5.6	*0.5*	0.7	*0.3*	1.2	*0.1*	0.8	*0.3*	2.8	*0.5*	1.0	*0.3*
18:3ω6,8,13	1.9	*0.8*	0.9	*0.4*	1.4	*0.5*	0.9	*0.4*	0.1	*0.0*	1.9	*0.1*	0.9	*0.2*	0.0	*0.0*
18:2ω(14,8 or 13,8)	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	0.5	*0.5*	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*
18:2ω6,9	24.0	*2.4*	2.0	*0.4*	10.1	*0.6*	0.9	*0.2*	1.9	*1.0*	2.3	*0.6*	8.2	*0.9*	1.0	*0.4*
18:1ω9	40.7	*1.3*	2.1	*0.7*	32.6	*3.1*	3.6	*0.8*	14.8	*0.8*	13.8	*4.2*	22.3	*1.0*	5.0	*1.3*
18:1ω7	24.8	*1.2*	2.0	*0.6*	20.1	*1.9*	4.5	*0.4*	12.6	*0.5*	12.5	*3.6*	29.2	*1.0*	10.0	*2.4*
18:1ω13	0.0	*0.0*	0.0	*0.0*	0.5	*0.5*	0.1	*0.1*	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*
18:1ω(10 or 11)	3.7	*0.3*	0.1	*0.1*	1.7	*0.9*	0.2	*0.1*	1.3	*0.3*	0.5	*0.2*	3.1	*0.2*	0.3	*0.1*
18:0	7.5	*0.3*	1.4	*0.3*	7.1	*0.6*	2.4	*0.3*	3.5	*0.2*	3.1	*0.4*	6.4	*0.5*	2.7	*0.4*
19:1ω6	2.0	*0.2*	0.0	*0.0*	2.0	*0.0*	0.2	*0.2*	1.1	*0.1*	0.7	*0.2*	3.6	*0.5*	1.0	*0.1*
10-Me-18:0	8.4	*0.7*	1.1	*0.2*	7.4	*0.7*	1.8	*0.3*	3.0	*0.2*	1.6	*0.4*	4.7	*0.7*	2.2	*0.4*
19:1ω8	0.5	*0.0*	0.0	*0.0*	0.3	*0.2*	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	0.1	*0.0*	0.0	*0.0*
19:0cy	23.8	*0.9*	1.7	*0.5*	23.2	*1.9*	3.4	*0.9*	15.3	*0.4*	11.3	*1.7*	21.6	*3.2*	7.4	*1.5*

16.5 Labile C proportion v MAT from all sites



17 Related publications

Published

Budge, K., Leifeld, J., Egli, M., Fuhrer, J. 2011. Soil microbial communities in sub)alpine grasslands indicate a moderate shift towards new environmental conditions 11 years after soil translocation. Soil Biology and Biochemistry 43, 1148-1154.

Budge, K., Leifeld, J., Hiltbrunner, E., M., Fuhrer, J. 2011. Alpine grassland soils contain large proportion of labile carbon but indicate long turnover times. Biogeosciences, doi: 10.5194/bg-8-1-2011

In progress

Authors: Budge, K., Leifeld, J., Fuhrer, J.
Title: “Soil organic carbon stocks but not labile fractions increase with altitude in Pyrenean limestone grassland”